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Growth and fattening stimulation in lambs and swine by certain androgenic and estrogenic compounds

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NOTE TO USERS

GROWTH AND FATTENING STIMULATION IN LAMBS AND SWINE BY CERTAIN ANDROGENIC AND ESTROGENIC COMPOUNDS

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by

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INTRODUCTION

Even though many of the details of the mechanisms by which hormones exert their effects, as well as the interrelationships that exist between the various endocrine glands, are not well defined, the fact is established that hormones exert effects that are sufficiently pronounced to be of interest to the livestock feeder. The role of the endocrine glands and the hormones they produce on the functions of the animal body has been recognized for years; however our knowledge of the effects of certain of these hormones on the body processes concerned with the economically important functions of growth, fattening, reproduction and lactation has accumulated, for the most part, in the last three decades.

The sex hormones and the hormones of the adrenal cortex are classified as steroid hormones. The sex hormones and the synthetic compounds which produce all or some of the same effects as certain of these natural hormones, have been used in recent research with meat producing animals. Of these, the compounds with estrogenic potency have shown the most promise. The ease of synthesis of these compounds makes them relatively inexpensive and their oral potency makes possible their daily administration as a part of the ration. The androgens and progesterone are also possibilities for

experimental exploration into ways of so altering the hormone balance that wholesome, nutritious, quality meat for our rapidly growing human population can be produced with greater economy per unit of feed.

To study several of these possibilities with sheep and with swine is the broad object of this research. To observe tissue and glandular development and the behavior of animals and thus gain additional information on the effects of sex hormone imbalance represents a secondary objective; and the determination of the wholesomeness and absolute safety of the meat produced for human consumption will serve as the first consideration in any recommendations made.

REVIEW OF LITERATURE

Work with the synthetic sex hormones in the growth and fattening of livestock was made possible in 1938 when Dodds et al. (34) synthesized the estrogenic compound diethylstilbestrol, hereinafter called stilbestrol. The synthesis of many other compounds with structure and action similar to or identical with certain of the natural hormones of the body followed (48). Among these are testosterone, estradiol, and progesterone all with identical structure to that of body hormones, and testosterone propionate and methyl testosterone with action similar to that of the natural androgen, testosterone. It is with this group of compounds that we are concerned in this investigation.

An excellent review of the effects of estrogens and androgens as they pertain to the production of meat was prepared for the National Research Council by Sykes et al. (78). Other reviews include that of estrogenic substances in livestock feeds and lamb nutrition by Story (77), the effects of stilbestrol on laboratory animals by Preston (67), and the ability of the male hormones or compounds with similar action to stimulate weight increases in small animals and in man by Kochakain (53).

Stilbestrol

Since 1949 it has been repeatedly demonstrated that stilbestrol administered as a pellet implant will significantly improve feed intake, rate of gain, and feed efficiency in beef cattle and usually in lambs, but will consistently lower carcass grade. This has been shown with lambs by Andrews et al. (2), Pope et al. (66), O'Mary et al. (61, 62), Means et al. (56), Jordan et al. (49, 50), and by Richard and Dinnuson (69) who observed that the improvement in rate of gain and feed efficiency was not dependent upon increased feed intake. Bell et al. (10) report similar results for rate of gain and carcass grades, but that a number of the lambs were lost in the group implanted with stilbestrol from prolapse of the rectum and from excessive swelling and inflammation in the rectal region. Stilbestrol treatment produced enlargement of the seminal vesicles, bladder, ampullae, uretha, prostate gland, and bulbourethral glands. This observation has been confirmed by Clegg et al. (31).

Similarly, in cattle, rate of gain and feed efficiency are improved and carcass quality lowered by the implantation of pellets of stilbestrol, Clegg and Cole (29), Andrews et al. (3), and Sykes et al. (78). Vaginal prolapse in

heifers, Clegg and Cole (29), has been credited to stilbestrol treatment as has mammary development, increased sexual activity, increase in teat length, and a relaxation in the loin region accompanied by an elevation in the tail head, Andrews et al. (3). Clegg and Cole (29) concluded from trials with cattle grazing irrigated pastures that a satisfactory response to stilbestrol implantation was dependent upon a high level of energy in the ration. Protein intake was not discussed.

Pellet implantations of stilbestrol with swine have been unimpressive in that undesirable side effects such as prolapse of the vagina, seediness of the belly, and riding and ranting have overshadowed any occasional slight improvement in rate of gain or in feed efficiency, Dinnuson et al. (33), Woehling et al. (85), and Sykes et al. (78).

That the administration of stilbestrol by pellet implantation increases nitrogen retention has been shown by Whitehair et al. (83), O'Mary et al. (61), and Jordan (49) for lambs; and by Clegg and Cole (29) for cattle. These workers, as did Galloway et al. (39) found no change in the digestibility of protein due to treatment.

The finding that the feeding of stilbestrol resulted in improvement in feed intake, weight gains, and feed efficiency equal or superior to that produced by stilbestrol

implantation without measurable adverse effects upon carcass quality and without observed undesirable side effects by Hale et al. (42) in two out of three trials with lambs, and Burroughs et al. (22) with beef steers, was accepted immediately by research workers and the livestock industry as having tremendous possibilities. The work with beef cattle showing that the feeding of approximately 10 mg. of stilbestrol per head daily to yearling steers being full-fed increased weight gains by as much as 35 percent and reduced the feed required per unit of gain by as much as 20 percent has been verified by a number of workers including Perry et al. (64), Bell et al. (9), Beeson et al. (8), and Baker and Jackson (5). These findings are not in exact agreement as to the treatment effect on carcass quality, and as to the absence of all of the side effects observed with stilbestrol pellet implantation. However, they do agree that any effect on carcass quality is slight and any resulting side effects are much less apparent and objectionable than are those resulting from the implantation of stilbestrol.

In 1954, the Food and Drug Administration, Department of Health, Education, and Welfare permitted the oral use of stilbestrol for beef cattle feeding through the use of carefully controlled premixes. This permit was based largely on the work of Burroughs et al. (22, 23) and subsequent work at

the Iowa station (24, 25) which established the economic advantage of the oral use of stilbestrol and also demonstrated that no estrogenic activity was present in the lean, fat, or liver tissues of cattle that had been fed stilbestrol, Preston et al. (68).

Published results of the oral use of stilbestrol in lamb rations include the previously mentioned summary of three trials by Hale et al. (42) who demonstrated, in two trials conducted during the winter and spring months, that the feeding of either 1.5, 2.0, or 3.0 mcg. of stilbestrol per pound of total ration increased weight gains and improved feed efficiency. The response in weight gains from the addition of 1.5 and 2.0 mcg. of stilbestrol to the ration was considered equivalent to the increased weight gains obtained with lambs administered 15 mg. of stilbestrol as a subcutaneous implant. Carcass quality was superior in the lambs fed stilbestrol. In a third trial, conducted during the summer months, no increase in weight gains or in feed efficiency resulted from the oral use of these levels of stilbestrol.

Acker et al. (1) fed stilbestrol at the rate of 0.5 mg. per pound of feed to lambs weighing 66 pounds initially for a period of 92 days and report a 15 percent improvement in rate of gain and feed efficiency over the control lambs.

Both dressing percent and carcass value were reduced by feeding stilbestrol. The lower carcass value was attributed to the fact that 26 percent of the treatment lambs graded as yearlings, whereas all of the control lambs yielded a lamb carcass.

Progesterone or Combinations Including Progesterone

O'Mary et al. (61) found, in two experiments, that the implanting of two 30 mg. pellets of progesterone had no effect on rate of gain or feed efficiency in lambs being finished for market. In the above experiment a combination of one 12 mg. pellet of stilbestrol and two 30 mg. pellets of progesterone were administered to ewe lambs with a resultant highly significant improvement in rate of gain. However, this increased gain was interpreted to be due to the stilbestrol rather than to the combination of stilbestrol and progesterone.

The subcutaneous implantation of 25 mg. of stilbestrol and 250 mg. of progesterone in wether and ewe lambs averaging 65 pounds initially is reported, by Galloway et al. (39), to have increased rate of gain, feed efficiency, carcass grades and dressing percentage. Bell et al. (10) report that lambs treated with implants of a combination

of stilbestrol and progesterone made larger gains than untreated control lambs but yielded 4 percent less and graded almost a full grade lower than the control lambs. Both the fat and the lean of the rib cuts contained more moisture than did similar cuts from the control lambs.

Michigan workers (58, 59) implanted 250 mg. progesterone and 10 mg. estradiol in wether and ewe lambs weighing 66 pounds initially and report a 27 percent increase in average daily gains and a 15 percent saving in feed required per unit of gain attributable to treatment. The implanted lambs produced carcasses of equal quality and both the dressing percentage and the percent of moisture in the fat were no different from the untreated control lambs. The implantations were made on the 35th day of a 63-day trial. A group of similar lambs implanted with 10 mg. of estradiol exhibited increased signs of estrus and there was much riding and restlessness as compared to the control group. In a second experiment designed to determine whether a more nearly ideal ratio of progesterone to estradiol existed, a combination of 100 mg. of progesterone and 10 mg. estradiol, implanted after six weeks of feeding, produced 42.5 percent faster gains on 24.6 percent less feed than the control lambs which gained at the very acceptable rate of .50 pound per head per day. This combination of progesterone and estradiol

was superior to that of 250 mg. progesterone and 10 mg. estradiol in rate of gain, efficiency of feed utilization, degree of finish, and the carcasses of this group contained less moisture in the external fat.

As an outgrowth of the above findings a request was made to the Food and Drug Administration, Department of Health, Education and Welfare for permission to use the 250 mg. of progesterone and 10 mg. of estradiol implant in lamb feeding. Permission was granted in 1954 and subsequently a commercial product, "Synovex", was made available to lamb feeders with a lamb dose comprising eight pellets advertised to contain 250 mg. progesterone and 10 mg. estradiol.

Acker et al. (1) implanted lambs averaging 66 pounds which were fed for 92 days with a progesterone-estradiol combination (Synovex) and report that the implanted lambs gained 55 percent faster than the untreated control lambs on 29 percent less feed per unit of gain. Bush et al. (27) found that ewe lambs implanted with progesterone and estradiol showed some increase in teat length and that a few of the lambs were in milk. Wether lambs showed some enlargement of the rudimentary teats.

Androgens or Combinations Including Androgens

Burris et al. (21) have been among the more successful

in using testosterone in finishing market animals. Unlike other workers, they used testosterone as such; whereas testosterone propionate or methyl testosterone are the synthetic androgens most frequently used. They report that the weekly intramuscular injection of 1 mg. of testosterone per kg. of body weight significantly improved the rate of gain of 500-pound beef calves. Average daily gain in pounds was 2.61 for the heifers treated with testosterone and 2.09 for the untreated control heifers, whereas the steers treated with testosterone gained an average of 2.74 and the control steers gained 2.65. The synthetic hormone was effective in increasing feed efficiency beyond its effect in increasing feed intake. The heifers treated with testosterone required 379 pounds of total digestible nutrients per 100 pounds of gain as compared to a requirement of 498 pounds of T.D.N. per 100 pounds of gain for the untreated control heifers. Heifers treated with testosterone had a slightly lower dressing percentage, a lower percentage of fat, a higher percentage of protein, and a higher percentage of round and chuck than the control heifers, while the steers treated with injections of testosterone were similar to the controls in these respects. The authors point out that part of the difference of the reduction in feed required per 100 pounds of gain in the group of heifers treated with

testosterone can readily be explained on the differences in the storage of fat in the treatment and control groups. Since fat contains 2.25 times as many calories per gram as does protein, lean animals storing low amounts of fat and high amounts of protein would be expected to gain weight on less feed than would animals storing high amounts of fat and low amounts of protein, other conditions being equal. The calves receiving testosterone developed a masculine appearance as well as patterns of masculine behavior which were not apparent in the control calves. The thyrotropic hormone content of the pituitaries from the calves treated with testosterone exceeded that of the untreated calves. The gonadotropic hormone content of the anterior pituitary was increased and corpus luteum formation was partially inhibited by treatment. The calves were each fed from a weight of approximately 500 pounds to a weight of 800 pounds.

Beeson et al. (8) compared the stimulation from methyl testosterone, stilbestrol and combinations of the two drugs by feeding them in an equalized food intake experiment with yearling steers. As compared to the control steers receiving no food additive, stilbestrol proved to be the greatest stimulator of weight gains. Methyl testosterone produced some response and a combination of the synthetic androgen and the synthetic estrogen was superior to the androgen

alone, but less effective than the stilbestrol alone. This trial indicates that the increase in weight gains resulting from the inclusion of stilbestrol or methyl testosterone is not the result of greater food intake. In working with androgens, Bogart et al. (14) compared the stimulation of testosterone and methyl androstenodiol. The compounds were administered intramuscularly to both steers and heifers in the form of aqueous suspensions of micropellets at the rate of 1 mg. per kg. of body weight. The calves injected with testosterone gained .40 pound more per head per day and required 100 pounds less T.D.N. per 100 pounds of gain than the untreated controls. Methyl androstenodiol, a non-masculazing hormone-like compound did not influence rate of gain or feed efficiency in either the steers or the heifers. The Oregon workers concluded that increased rate of gain and feed efficiency are apparently associated with the masculizing principle of the male hormone.

Estrogenic Activity of Edible Tissues

A review of work published prior to 1954 on the estrogenic activity of the edible tissues from meat animals and poultry that had been treated by stilbestrol implantation has been made by Stob et al. (75). Using the uterine

weight response of ovariectomized mice as a measure of estrogenic activity in tissues from cattle and sheep that had been implanted with stilbestrol they conclude that the amount of hormone present in beef muscle and liver does not exceed 0.01 mcg. per gram of dried tissue and 0.10 mcg. per gram of dried tissue in the case of sheep muscle. In the case of lambs the activity was found in the lean tissue; whereas with beef the activity was found in both liver and lean.

Whiting et al. (84) using female weanling rats as the assay animal report that kidney fat from lambs treated by stilbestrol pellet implantation showed considerable activity; whereas muscle tissue showed but little activity in one experiment and more in another trial. Liver extract showed no response, but liver residue showed evidence of estrogenic activity in one of two trials. The greatest activity found represented 6.6 mcg. of stilbestrol per 100 grams of kidney fat. No response was detectable in the tissues from the untreated control lambs.

The above findings with tissues from cattle and lambs treated by stilbestrol pellet implantation is in contrast to the finding that no estrogenic activity has been detected by biological assay in the tissues of steers fed stilbestrol - Burroughs et al. (24) with beef liver, lean, and fat from cattle that had been off feed a short time before slaughter;

and Perry et al. (63) with beef neck meat from animals taken off feed containing stilbestrol from one to seven days prior to slaughter.

Similarly, neither Braude (17) nor Beeson et al. (7) could detect estrogenic activity, by biological assay, in pork (fat and lean) from pigs fed stilbestrol at rates of 2 mg. per pig daily or 40 mg. per pig daily; whereas Taylor and Gordon (79) report that a heat stable estrogenic substance was present in the carcasses of pigs fed stilbestrol which was not present in the carcasses from the control pigs as determined by the uterine weight response of immature female rats. The pigs, in this case, were fed 6 mg. stilbestrol per head per day, but the age of the rats and the description of the meat fed the rats are not given.

Preston et al. (68) concluded that no stilbestrol residues were detectable in any beef tissues including lean, fat, liver, heart, kidney, and offal tissues including beef tripe, intestines, lungs and spleen from cattle that had been fed stilbestrol during the feeding period and up to the time of being loaded on trucks for shipment to market. They concluded that the bio-assay technique employing the use of immature female mice was sufficiently sensitive to detect as little as 0.003 mcg. of stilbestrol per gram of diet. This represents a sensitivity for approximately two parts of stilbestrol per billion parts of fresh tissue.

EXPERIMENTAL

Part I. Studies with Swine

Method and materials

Swine Experiment 622. To study the effects of feeding different levels of stilbestrol to growing-finishing pigs, ten ration treatments were assigned at random, in each of two replicates, to ten pens of four pigs each. The 80 pigs selected for the trial were grouped into two weight brackets and allotted from outcome groups by weight and sex. Four pigs were used per pen to an average weight of 100 pounds after which time one barrow and one gilt, determined by previous random selection, were terminated to prevent overcrowding. The pigs varied initially from 25 to 55 pounds with an average age of 61.5 days. Average starting weights of the groups in the various treatments ranged from 41 to 45 pounds for the heavy replicate and from 30 to 34.75 pounds for the light replicate. The pigs were out of crossbred (Poland China X Landrace X Duroc) sows and were by either Landrace or Duroc boars.

All pigs were wormed with a 0.5 percent sodium fluoride ration and sprayed with benzene hexachloride for external

parasites immediately before the start of the experiment. Feed was allowed ad libitum, water was provided in troughs, and the pigs were confined to indoor concrete pens. The pigs were weighed individually and the feed weighed back each week until an average pen weight of 100 pounds was reached and then each two weeks for the duration of the trial. Each pig was removed from the experiment at weights of approximately 100 or 200 pounds.

The ration ingredients are shown in Table 1. The treatment rations varied only in the level of stilbestrol, these levels being 0, 5, 10, 20, 40, 80, 160, 320, 640, and 1280 mcg. per pound of ration. Stilbestrol premixes were prepared by dissolving a known quantity of stilbestrol in 95 percent ethyl alcohol, mixing this solution, by hand, in 5 pounds of soybean oil meal, then incorporating this batch with a larger quantity of soybean oil meal by mechanical mixing.

The soybean oil meal was a blend of equal parts of three manufacturers' hexane solvent extracted meal. Protein levels used were 16 percent up to 75 pounds, 14 percent from 75 to 150 pounds, and 12 percent from 150 to 200 pounds. A chlortetracycline concentrate was added to the ration so as to supply 5 mg. of chlortetracycline per pound of ration.

As each pig was weighed out of the trial at approximately 200 pounds a measure of the depth of backfat hereinafter

Table 1. Composition of the 16 percent protein basal ration - Swine Experiment 622

	(percent)
Ground yellow corn	77.45
Solvent soybean oil meal (blended)	18.00
DL methionine	0.05
Dicalcium phosphate	1.20
Calcium carbonate	0.70
Iodized salt	0.50
Trace mineral mixture[a]	0.10
Vitamin and antibiotic premix 622-16[b]	2.00
Stilbestrol premix	--
	100.00
Calculated analysis	
Protein	16.08
Fat	3.24
Crude fiber	3.24
Calcium	0.701
Phosphorus	0.499
Vitamin A, I.U. per lb.	3000.00
Vitamin D_2, I.U. per lb.	400.00
Riboflavin, mg. per lb.	2.11
Niacin, mg. per lb.	23.80
Pantothenic acid, mg. per lb.	7.80
Choline, mg. per lb.	805.00
Vitamin B_{12}, mcg. per lb.	5.00

[a]Contributed in ppm to the ration: iron, 70; copper, 4.8; cobalt, 1.6; manganese, 59; zinc, 4.4; potassium, 76.

[b]Vitamin and antibiotic premix 622-16 supplied the following amounts of vitamins per pound of ration:

Vitamin A	2225.0 I.U.
Vitamin D_2	400.0 I.U.
Riboflavin	1.5 mg.
Pantothenic acid	5.0 mg.
Niacin	15.0 mg.
Choline	400.0 mg.
Vitamin B_{12}	5.0 mcg.

called the "live probe" was taken at two locations, as described by Hazel and Kline (45).

Immediately before slaughter a measure of the cervical opening was taken by inserting the largest specially prepared pyrex glass rod that would pass through the cervix with moderate pressure. The glass rods ranged in diameter from 2 to 24 mm., were rounded at the ends by fire polishing, and were 13 inches in length.

The gilts were slaughtered through the Iowa State College meats laboratory within three days after reaching the termination weight of approximately 200 pounds. The barrows were sold on the local market. Dressing percent was calculated from the chilled carcass weight, taken after 72 hours of chilling, and the live weight taken immediately before slaughter with the pigs being allowed feed and water up to the time of this weight determination.

Weight of the fresh liver was taken at the time of slaughter and the livers from all gilts on the 0, 10, 160, and 1280 mcg. of stilbestrol per pound of ration treatments were frozen for later use in biological assay for estrogenic activity. A portion of the ham end of the loin was separated into separable fat and lean with both portions being saved for mouse assay.

An estimate of the size of the pelvic inlet was taken

after the carcasses were split. The left side was used throughout with the conjugate and transverse diameters of the pelvic inlet calculated.

The entire reproductive tract was obtained from the killing floor, trimmed of adhering adipose tissue and weighed with the ovaries attached. The ovaries were then removed, weighed as quickly as possible and a count made of the follicles and/or corpora leutea present.

Swine Experiment 637. This trial was essentially a repetition of Experiment 622, except that three replicates with two pigs per pen were used throughout the trial. The same procedure of random allotment from outcome groups by weight and sex was used, but the experimental animals allowed for uniformity of breeding within the various replicates. Thus, two replicates, heavy and light, of pigs by Landrace boars and out of crossbred (Poland China X Landrace X Duroc) sows, and one replicate by Duroc boars and out of crossbred sows as described above were used. Average starting weights on the various treatments ranged from 36 to 41 pounds for the heavy replicate representing pigs by Landrace boars; 27 to 32 pounds for the light replicate for pigs by Landrace sires; and 27.5 to 33.5 pounds for the pigs by the Duroc sires that made up the third replicate. Feeding, watering and housing were handled as in the first

trial. The pigs were weighed individually and the feed weighed back each 14 days.

The ration ingredients are shown in Table 2. The stilbestrol premix was the same as in Experiment 622 except that ground shelled corn was used as the carrier in place of soybean oil meal. The protein levels used were 14 percent up to 100 pounds and 10 percent from 100 pounds to a termination weight of approximately 200 pounds. Chlortetracycline was again added at the rate of 5 mg. per pound of ration though from a different commercial source.

All of the gilts were slaughtered with those in one replicate allowed the experimental ration up to the time of slaughter; those in another replicate had the treatment ration replaced by the basal ration 24 hours before slaughter; and those on the third replicate changed to the basal ration 48 hours before slaughter. At the time of ration change an iron oxide marker was administered in order to determine that the experimental ration had cleared the alimentary tract by the time of slaughter.

Dressing percent was calculated from the chilled carcass weight and the last experimental weight, as the treatment of the gilts from the time they were weighed out of the experiment to the time of slaughter was not uniform for all animals.

Table 2. Composition of the 14 percent protein basal ration - Swine Experiment 637

	(percent)
Ground yellow corn	82.60
Solvent soybean oil meal (blended)	12.50
Dicalcium phosphate	1.70
Calcium carbonate	0.60
Iodized salt	0.50
Trace mineral mixture[a]	0.10
Vitamin and antibiotic premix 637-14[b]	2.00
Stilbestrol premix	--
	100.00
Calculated analysis	
Protein	13.90
Fat	2.62
Crude fiber	3.00
Calcium	0.71
Phosphorus	0.50
Vitamin A, I.U. per lb.	3000.00
Vitamin D_2, I.U. per lb.	400.00
Riboflavin, mg. per lb.	1.50
Niacin, mg. per lb.	15.00
Pantothenic acid, mg. per lb.	5.00
Choline, mg. per lb.	450.00
Vitamin B_{12}, mcg. per lb.	5.00

[a]Explained in Table 1.

[b]Vitamin and antibiotic premix 637-14 supplied the following amounts of vitamins per pound of ration:

Vitamin A	2174.00 I.U.
Vitamin D_2	400.00 I.U.
Riboflavin	0.94 mg.
Pantothenic acid	2.385 mg.
Niacin	6.441 mg.
Choline	103.60 mg.
Vitamin B_{12}	5.00 mcg.

To determine if the fat, liver, and lean tissue from gilts fed stilbestrol under the conditions of this experiment contained estrogenic activity, 80 ration treatments were assigned at random, in each of two replicates, to 160 pens of five mice each. Immature albino Swiss female mice from 8 to 10 grams in weight of the Harlan Webster Swiss strain were used. The mice were contained in metal cages, bedded with wood shavings, housed in an air conditioned room at a temperature of 70 degrees Fahrenheit, provided water ad libitum with tube-type water bottles and hand-fed twice daily. All pens concerned in a certain tissue assay were fed the same per mouse allowance daily and all refused feed was weighed back. A basal ration of 74 percent finely ground and sifted corn, 20 percent dry skim milk, 4 percent corn oil, and 2 percent of a commercial salt mixture prepared for small animal use was used.

The lean and liver tissues to be assayed were dried in a forced air oven to constant weight, ground as fine as possible in a Wiley mill, then mixed with mortar and pestle with the basal mouse diet. The fat was dried as described above. The percent of the different tissues mixed with the basal mouse diet to provide the experimental rations was: lean 50, liver 40, and fat 30. Stilbestrol was added to both the standard and unknown ration at levels of 0, .005,

.010, and .020 mcg. per gram of mouse ration. The stilbestrol to be added to the rations was dissolved in ethanol and freshly prepared in stock solutions so that 10. ml. of solution would deliver the amount of stilbestrol needed for each 200 grams of mouse diet.

The experimental period was six days for the lean and fat assay and seven days for the liver assay. On the morning following the last experimental day the mice were destroyed, the uteri dissected and fixed in Bouin's fluid for 24 hours, All uteri were then trimmed uniformly, freed of adhering tissue, pressed dry against several thicknesses of filter paper, and weighed on a Roller-Smith torsion balance of 500 mg. capacity and calibrated in 2 mcg. intervals.

The method of estimating the quantity of stilbestrol, or activity equivalent thereto, was that of parallel lines as given by Finney (36) and adapted for this particular type of assay by Homeyer (47). For all assays analyzed the necessary tests of statistical validity of the parallel line assay were made.

The quantity of stilbestrol, above the known quantity added for the assays, in the unknown samples was estimated by

$$M = \frac{\bar{y}_u - \bar{y}_s}{b} \text{ , where}$$

$\bar{y}_u$ represents the average of the mean uterine weights per pen of all pens fed the unknown,

$\bar{y}_s$ represents the average of the mean uterine weights per pen of all pens fed the standard, and

b represents the combined regression coefficient for the standard and unknown response curves.

The confidence, or fiducial, limits of M were computed, and the minimum value of M required to be statistically significant at P = .05 was computed according to Homeyer (47). The value of M, under these conditions, measures the estrogenic activity equivalent to mcg. of stilbestrol per gram of mouse diet. The value obtained can then be converted to the tissue component of the ration, then to the fresh tissue basis from the values determined for moisture content of the tissues from the drying procedure previously described.

With only a few exceptions, the several groups of data were analyzed statistically. The mean squares for these analyses are shown in the Appendix, Tables 27 to 41.

In either the Results or Discussion sections, the use of the term significance means statistical significance at P = .05 or less; in some cases it is P = .01. Several pigs were lost in the experiment, and for these, missing values were calculated by the method of Snedecor (74).

Results

Weight gains and feed data. Data for daily gain, feed intake, and feed required per pound of gain are shown in Tables 3 and 4. These data for average daily gain and feed required per pound of gain are also presented graphically in Figures 1 and 2. No differences in rate of gain or feed efficiency, of statistical significance, resulted from the feeding of 5 to 1280 mcg. of stilbestrol per pound of ration. This was true for both the initial to 100-pound and for the initial to 200-pound periods.

A trend was observed in Experiment 622 indicating that gains were stimulated slightly at the lower levels of either 5 or 10 mcg. of stilbestrol per pound of feed, and even more at the 160 mcg. per pound of feed level. This indication of stimulation at low levels agrees in part with the work of Catron *et al.* (28) who observed more improvement in rate of gain at a level of 10 mcg. per pound of feed than at levels of either 5 or 100 mcg. of stilbestrol per pound of ration. In Experiment 637 no evidence of stimulation at the lower levels was apparent, but the 160 mcg. level was again one of the two best levels being exceeded only by the level of 80 mcg. in rate of gain. No sex differences due to treatment were significant, although the 160 mcg. level produced nearly equal performance in both barrows and gilts. No

Table 3. Summary of growth and feed data - Swine Experiment 622

Stilbestrol mcg./pound basal ration	37.6 to 100 lbs.[a]					37.6 to 200 lbs.[b]				
	Average daily gain			Feed per day	Feed per lb. gain	Average daily gain[c]			Feed[d] per day	Feed[d] per lb. gain
	Barrows	Gilts	Av.			Barrows	Gilts	Av.		
					(pounds)					
0	1.36[e]	1.45[e]	1.40	3.81	2.72	1.62	1.72	1.67	5.51	3.30
5	1.38	1.44	1.41	3.89	2.76	1.76	1.74	1.74	5.86	3.37
10	1.58	1.37	1.47	3.88	2.64	1.88	1.61	1.74	5.50	3.16
20	1.34	1.26[e]	1.30	3.61	2.78	1.76	1.59	1.67	5.46	3.27
40	1.46	1.30	1.38	3.85	2.79	1.63	1.62	1.62	5.38	3.32
80	1.43	1.42[e]	1.42	4.17	2.94	1.69	1.66	1.67	5.81	3.48
160	1.45	1.41	1.43	4.02	2.81	1.84	1.86	1.86	5.77	3.10
320	1.29	1.42	1.36	3.64	2.68	1.74	1.70	1.72	5.57	3.24
640	1.35	1.33	1.34	3.62	2.70	1.78	1.80	1.79	5.64	3.15
1280	1.44	1.33	1.38	3.76	2.66	1.58	1.70	1.64	5.38	3.28
Average	1.41	1.37	1.39	3.82	2.75	1.73	1.70	1.71	5.58	3.27

[a]Four pigs per pen. Two pens per level. Treatment effects not significant at P = .05 or less.

[b]Treatment effects not significant at P = .05 or less.

[c]Two pigs per pen. Two pens per level.

[d]Two pens of four pigs each to 100 lbs., then two pens of two pigs each.

[e]Estimated values for pigs removed for causes not due to experimental treatment.

Table 4. Summary of growth and feed data - Swine Experiment 637[a]

Stilbestrol mcg./pound basal ration	33 to 100 lbs.[b]					33 to 200 lbs.[b]				
	Average daily gain			Feed per day	Feed per lb. gain	Average daily gain			Feed per day	Feed per lb. gain
	Barrows	Gilts	Av.			Barrows	Gilts	Av.		
					(pounds)					
0	1.49	1.47	1.48	4.29	2.90	1.65	1.60	1.63	6.03	3.70
5	1.51	1.37[c]	1.44	4.22	2.98	1.73[c]	1.56	1.65	6.10	3.70
10	1.56	1.34	1.45	4.26	2.93	1.69	1.55	1.62	6.05	3.74
20	1.43	1.31	1.37	4.04	2.94	1.63	1.52	1.58	5.87	3.72
40	1.44[c]	1.45	1.44	4.05	2.84	1.60[c]	1.61	1.61	5.78	3.78
80	1.65	1.23	1.44	4.14	2.86	1.87	1.60	1.74	6.13	3.55
160	1.53	1.53	1.53	4.29	2.81	1.71	1.65	1.68	5.96	3.58
320	1.43	1.50	1.47	4.11	2.81	1.57	1.60	1.59	5.78	3.61
640	1.31	1.33	1.32	4.28	3.24	1.60	1.56	1.58	6.52	4.11
1280	1.51	1.31	1.41	4.21	2.99	1.71	1.56	1.64	6.18	3.78
Average	1.49	1.38	1.44	4.19	2.93	1.67	1.58	1.63	6.04	3.73

[a]Treatment effect not significant at P = .05 or less.

[b]Two pigs per pen. Three pens per level.

[c]Estimated values for pigs removed for causes not due to experimental treatment.

Figure 1. Relationship of level of stilbestrol to average daily gain and feed required per pound of gain - Swine Experiment 622

EXPERIMENT 622

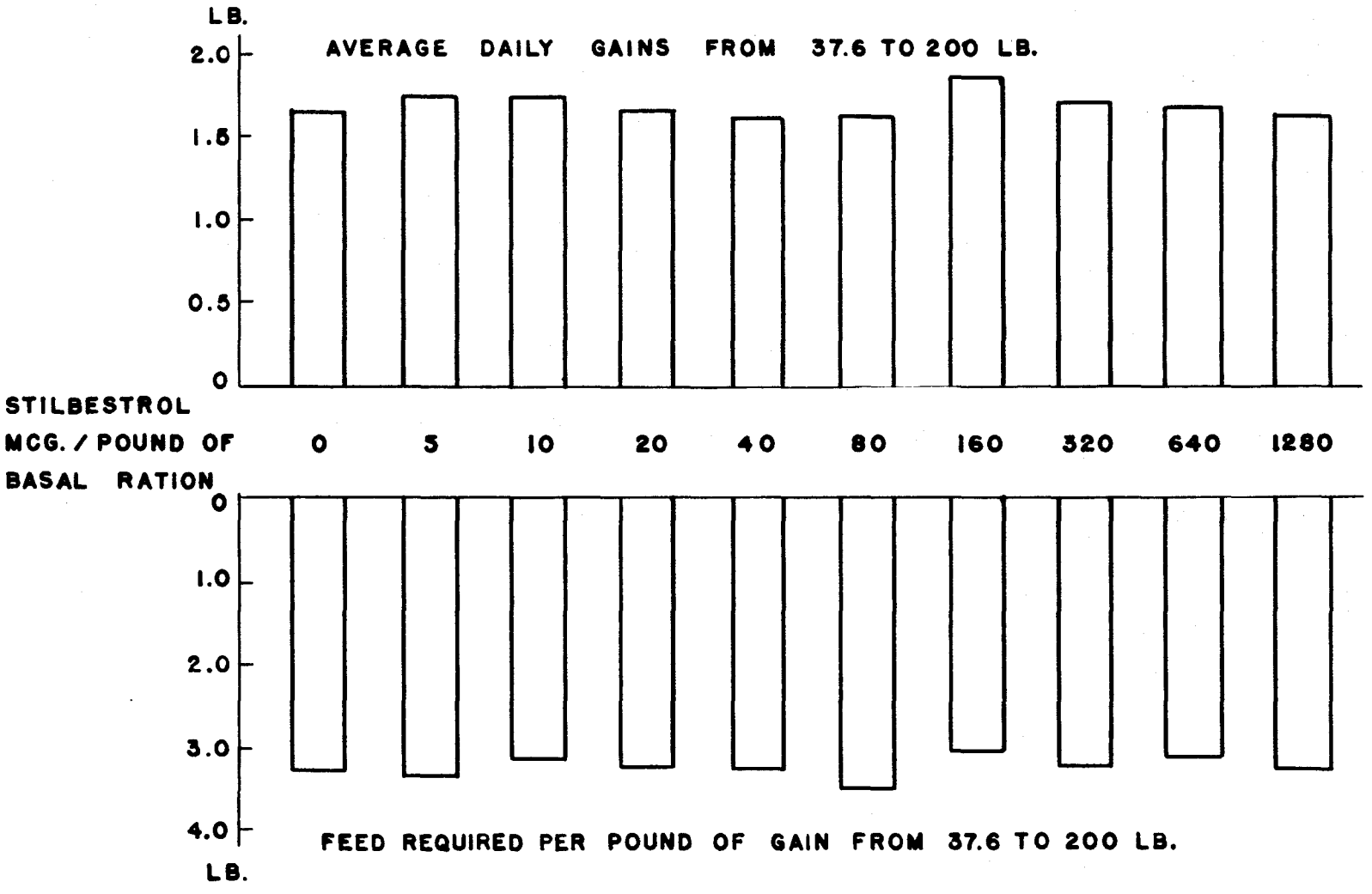
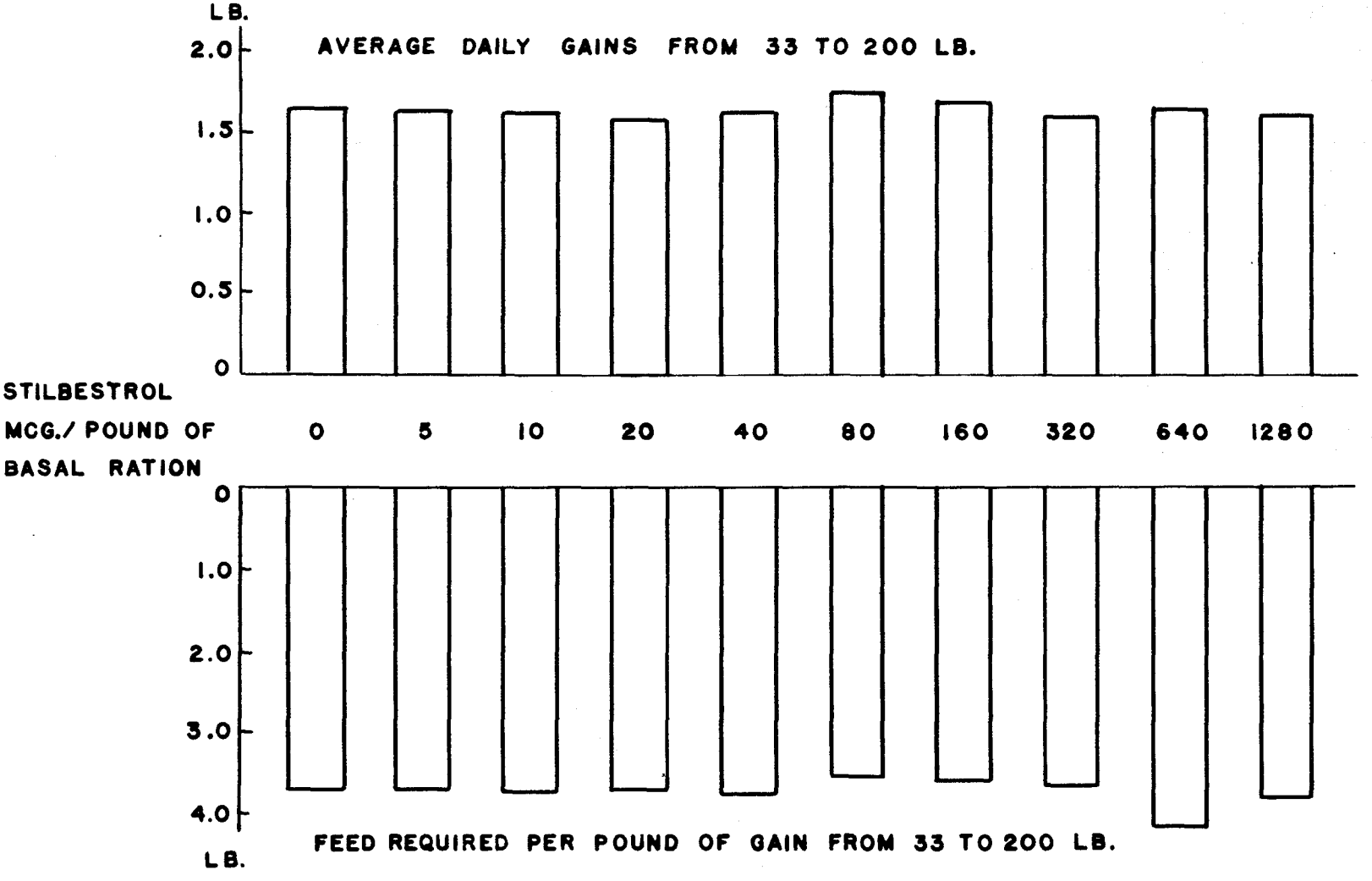


Figure 2. Relationship of level of stilbestrol to average daily gain and feed required per pound of gain - Swine Experiment 637

EXPERIMENT 637



other level showed this trend to this degree. Although slight differences in feed consumed per day exist, these data show no consistent trend or statistical significance.

Live probe and carcass characteristics. The data for live probe measurements at 200 pounds are presented in Table 5. The differences indicate no definite trend and are not significant. The pigs fed 320 mcg. of stilbestrol per

Table 5. Live probe measurements - Swine Experiments 622 and 637

Stilbestrol mcg./pound basal ration	Experiment 622[a] 37.6 to 200 lbs.[b]			Experiment 637[a] 33 to 200 lbs.[c]		
	Barrows	Gilts	Av.	Barrows	Gilts	Av.
			(inches)			
0	1.3	1.4	1.3	1.6	1.6	1.6
5	1.5	1.5	1.5	1.6	1.5	1.6
10	1.6	1.5	1.6	1.4	1.5	1.4
20	1.5	1.4	1.5	1.7	1.3	1.5
40	1.4	1.6	1.5	1.6	1.6	1.6
80	1.7	1.5	1.6	1.7	1.6	1.7
160	1.7	1.3	1.5	1.6	1.4	1.5
320	1.4	1.4	1.4	1.4	1.5	1.4
640	1.4	1.5	1.5	1.4	1.4	1.4
1280	1.7	1.6	1.6	1.5	1.6	1.6
Average	1.5	1.5	1.5	1.6	1.5	1.6

[a]Treatment effect not significant at P = .05 or less.

[b]Two pigs per pen. Two pens per level.

[c]Two pigs per pen. Three pens per level.

pound of feed actually appeared to carry less finish as judged on foot and the live probe measurements do not refute this observation. Again, no sex difference due to treatment was apparent nor was it shown by statistical analysis.

Table 6 lists the data for cooler shrink and carcass yield. These data show considerable variation, but lack significance statistically. All carcasses were watched

Table 6. Dressing percent and carcass shrink in chilling 72 hours - Swine Experiments 622 and 637

Stilbestrol mcg./pound basal ration	Experiment 622[a]		Experiment 637[b]	
	Cooler shrink[c]	Dressing percent[c]	Cooler shrink[c]	Dressing percent[c]
	(percent)		(percent)	
0	3.70	75.2	2.56	74.6
5	3.94	71.7	3.01	73.4
10	4.50	71.4	2.64	73.0
20	3.06	75.1	2.66	74.7
40	3.45	73.3	2.60	74.3
80	3.89	71.7	3.18	73.3
160	2.76	70.6	2.51	74.9
320	3.30	71.8	2.54	74.0
640	3.55	72.7	2.64	73.0
1280	3.24	72.8	2.80	72.9
Average	3.54	72.6	2.71	73.7

[a]One gilt per pen. Two pens per level.

[b]One gilt per pen. Three pens per level.

[c]Treatment effect not significant at P = .05 or less.

closely on the cutting floor for evidence of any observable differences in quality, but none were apparent that could be attributed to treatment.

Other observable effects. The feeding of stilbestrol at a level of 320 mcg. per pound of feed, or at any higher level used, produced gross enlargement of the vulva by the third day of the trial. By the eighth day of the trial, the vulvas of those gilts on the 160 mcg. per pound level were slightly enlarged. Figures 3-7 show these effects as of the eighth day of Experiment 622. The gilts in Experiment 637 followed the same pattern. Pictures of gilts on various levels taken at the end of the experiment are shown in Figures 8-13. It can be observed that the division for levels causing enlargement of the vulva is between 80 and 100 mcg. of stilbestrol per pound of feed. No level below 160 mcg. caused either enlargement of the vulva or teats, whereas levels of 160 mcg. or more of stilbestrol per pound of feed increased the size of the vulva.

Enlargement of the teats in gilts and of the rudimentary teats in barrows occurred at the 160 mcg. level or above, but did not develop until after approximately 30 days of experimental treatment. Teat size, unlike vulva size, did not seem to be graded as to level of stilbestrol for those levels that stimulated the mammary gland. Whereas

the enlarged teats were quite apparent in the warm carcass they were hard to detect in the chilled carcass.

The data for the diameter of the cervix and weight of the reproductive tract are presented in Table 7. These data for cervix diameter are shown graphically in Figure 14. The feeding of stilbestrol at levels of 5 to 1280 mcg. per pound of feed produced a significant increase in the diameter of the cervix. The diameter size increased with size of dose up to the 10 mcg. per pound level, then in general plateaued through levels of 10, 20, 40, 80, and 160 mcg. per pound and increased again through levels of 320, 640, and 1280 mcg. of stilbestrol per pound of feed. A comparison of these data with those for the size of the reproductive tract indicates that this increase in the diameter of the cervix is not a mere function of increased size of tract, although it is true that the highest levels of stilbestrol produced the largest reproductive tracts.

The size of the reproductive tract was increased significantly, but not uniformly, by treatment, and in Experiment 637 the reproductive tracts of pigs by Duroc sires were increased significantly more than were those by Landrace boars; all pigs being from crossbred (Poland China X Landrace X Duroc) sows. Figures 15 to 20 show the reproductive tracts from gilts fed five levels of stilbestrol.

The effect of stilbestrol on the vulva of gilts as of the eighth day - Swine Experiment 622

Figure 3. Gilt 9526 fed the basal ration

Figure 4. Gilt 9640 fed 160 mcg. stilbestrol per pound of ration

Figure 5. Gilt 9505 fed 320 mcg. stilbestrol per pound of ration

Figure 6. Gilt 9476 fed 640 mcg. stilbestrol per pound of ration

Figure 7. Gilt 9436 fed 1280 mcg. stilbestrol per pound of ration

FIGURE 3

FIGURE 4

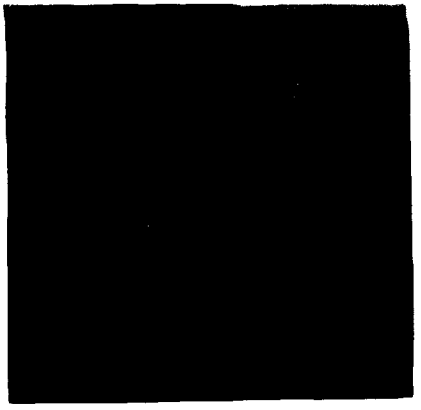


FIGURE 5

FIGURE 6

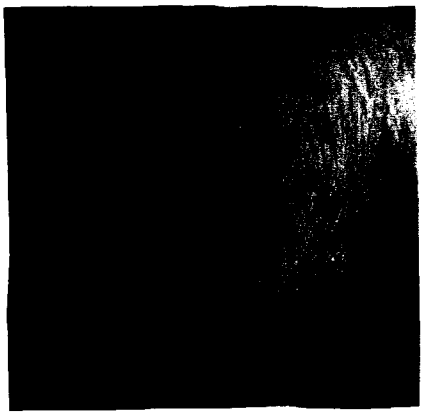


FIGURE 7

Table 7. Level of stilbestrol and the diameter of the cervix and weight of the reproductive tract - Swine Experiments 622[a] and 637[b]

Stilbestrol mcg./pound basal ration	Experiment 622		Experiment 637	
	Cervix diameter[c]	Weight of reproductive tract[d]	Cervix diameter[c]	Weight of reproductive tract[d]
	(millimeters)	(grams)	(millimeters)	(grams)
0	2.0	115.50	4.0	183.20
5	6.5	132.45	4.5	152.67
10	13.0	207.00	9.0	170.77
20	12.0	104.00	9.0	135.76
40	14.0	135.50	9.3	169.43
80	13.0	130.45	8.3	157.67
160	14.0	184.00	12.0	130.70
320	18.0	159.25	12.7	185.90
640	18.0	170.45	13.3	253.80
1280	15.5	235.45	15.3	276.53
Average	12.6	157.30	9.74	183.90

[a,b]Explained in Table 6.

[c]Linear component significant at P = .01.

[d]Linear component significant at P = .05.

The effect of stilbestrol on the vulva of gilts at a weight of 200 pounds - Swine Experiment 622

Figure 8. Gilt 9505 fed the basal ration

Figure 9. Gilt 9439 fed 10 mcg. stilbestrol per pound of ration

Figure 10. Gilt 9438 fed 80 mcg. stilbestrol per pound of ration

Figure 11. Gilt 9279 fed 160 mcg. stilbestrol per pound of ration

Figure 12. Gilt 9362 fed 640 mcg. stilbestrol per pound of ration

Figure 13. Gilt 9436 fed 1280 mcg. stilbestrol per pound of ration

(The gilts in Figures 9, 10, and 13 are littermates.)

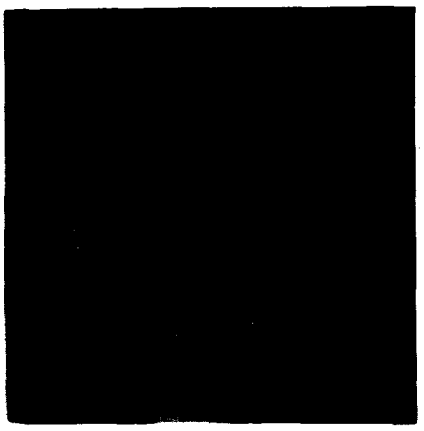


FIGURE 8

FIGURE 9

FIGURE 10

FIGURE 11

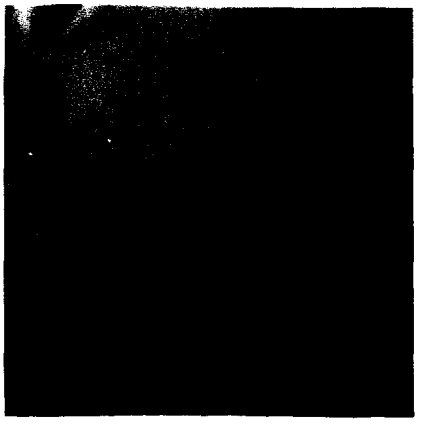


FIGURE 12

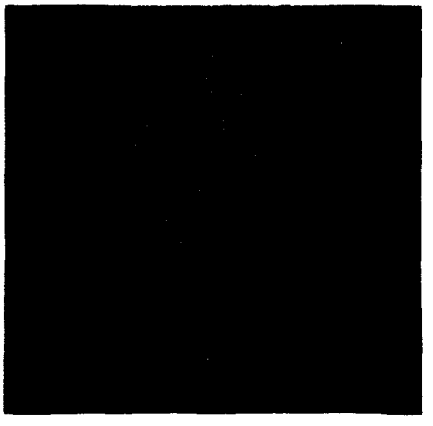
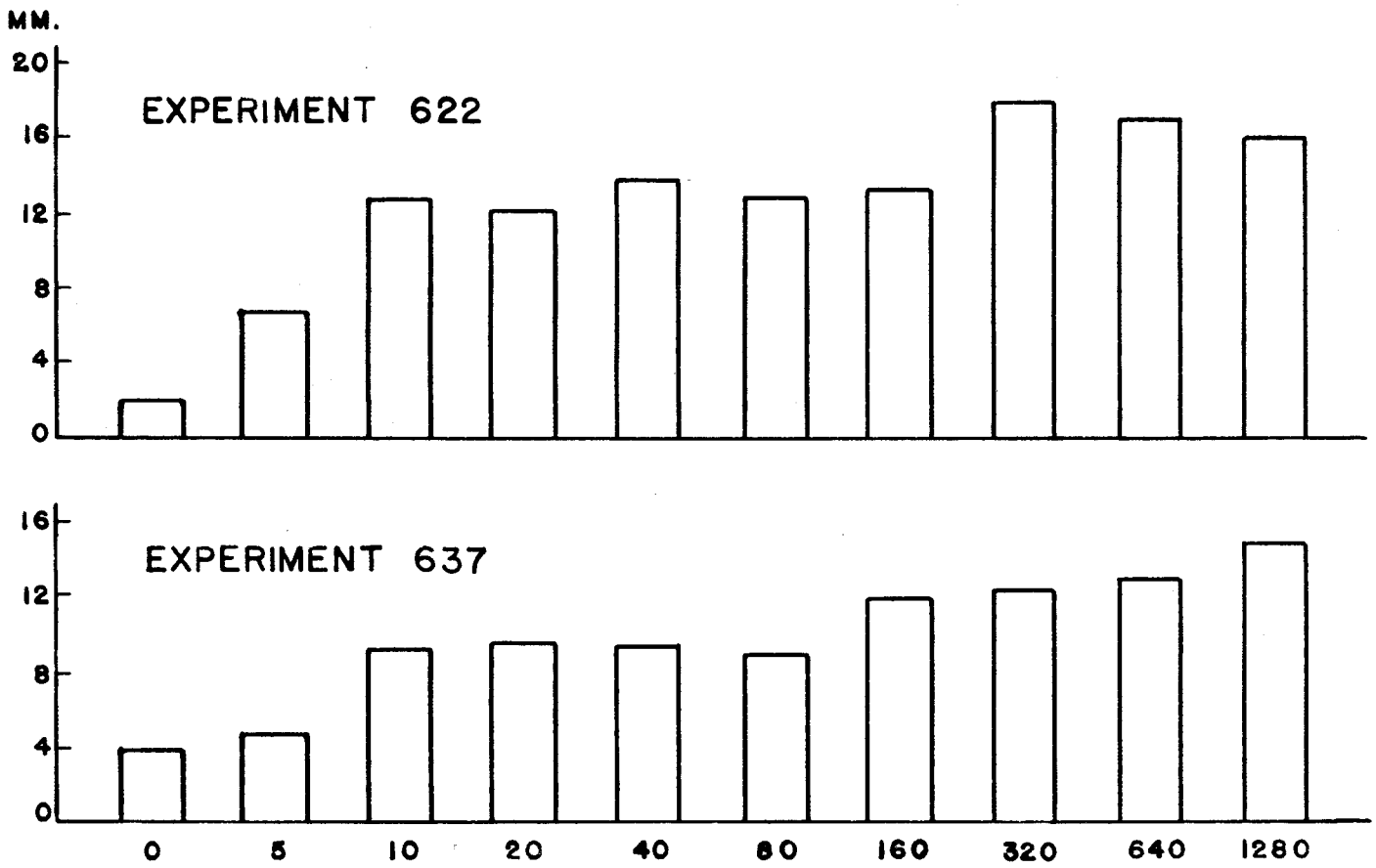


FIGURE 13

Figure 14. Relationship of the level of stilbestrol to the diameter of the cervix



AVERAGE DIAMETER OF THE CERVIX

Relationship of level of stilbestrol and size of the reproductive tract - Swine Experiment 637

Figure 15. Portion of the reproductive tract from Gilt 646 fed the basal ration - weight of the entire tract 193.9 grams

Figure 16. Portion of the reproductive tract of Gilt 563 fed 10 mcg. stilbestrol per pound of ration - weight of the entire tract 276.0 grams

FIGURE 15

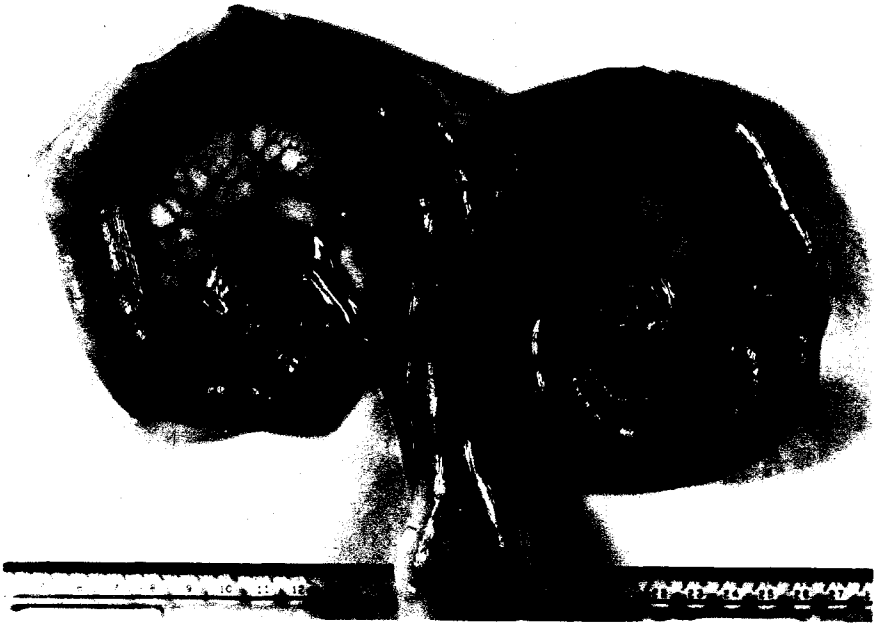


FIGURE 16

Relationship of level of stilbestrol and size of the reproductive tract - Swine Experiment 637

Figure 17. Portion of the reproductive tract from Gilt 645 fed 80 mcg. stilbestrol per pound of ration - weight of the entire tract 226.0 grams

Figure 18. Portion of the reproductive tract from Gilt 576 fed 160 mcg. stilbestrol per pound of ration - weight of the entire tract 172.9 grams

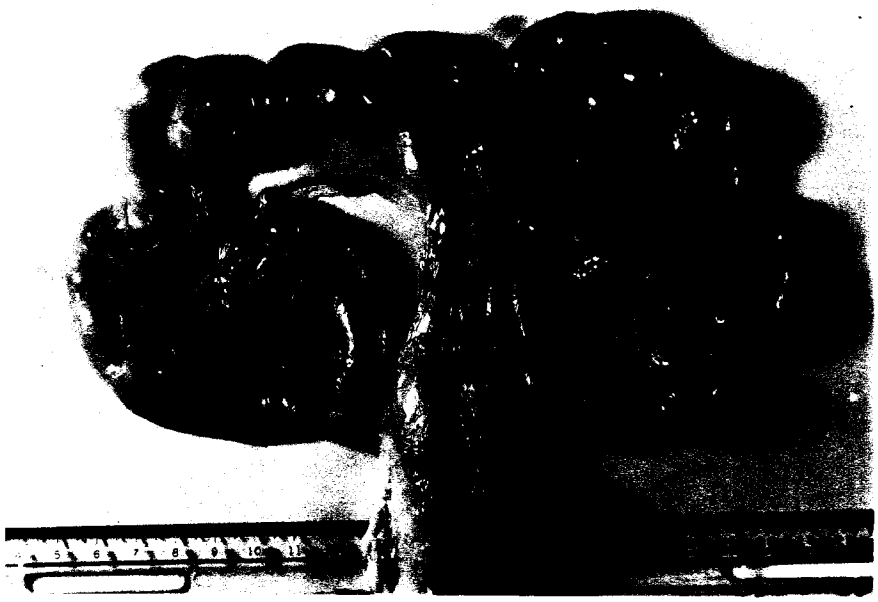


FIGURE 17

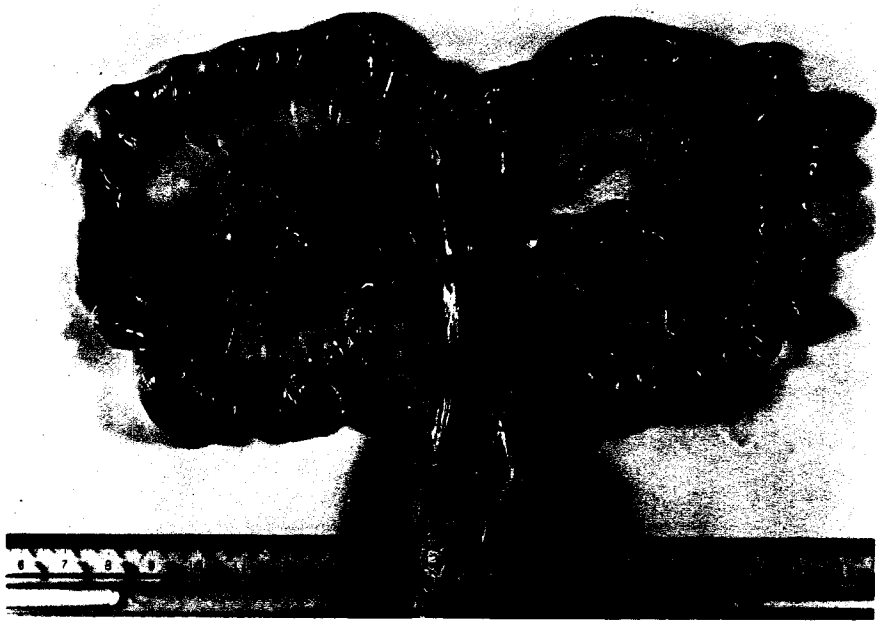


FIGURE 18

Relationship of level of stilbestrol and size of the reproductive tract - Swine Experiment 637

Figure 19. Portion of the reproductive tract of Gilt 564 fed 640 mcg. stilbestrol per pound of ration - weight of the entire tract 424 grams

Figure 20. Portion of the reproductive tract of Gilt 577 fed 1280 mcg. stilbestrol per pound of ration - weight of the entire tract 350 grams

FIGURE 19

FIGURE 20

They show, too, as does Table 8, and Figures 21 to 25, the significant reduction in ovary size at the three higher levels of stilbestrol feeding. Again the 160 mcg. of stilbestrol per pound of ration level proved to be the breaking point above which ovary size was greatly reduced and the number of follicles reduced to zero in one experiment and to zero to two in the other experiment.

Table 8. Weight of the ovaries and number of follicles per ovary - Swine Experiments 622[a] and 637[b]

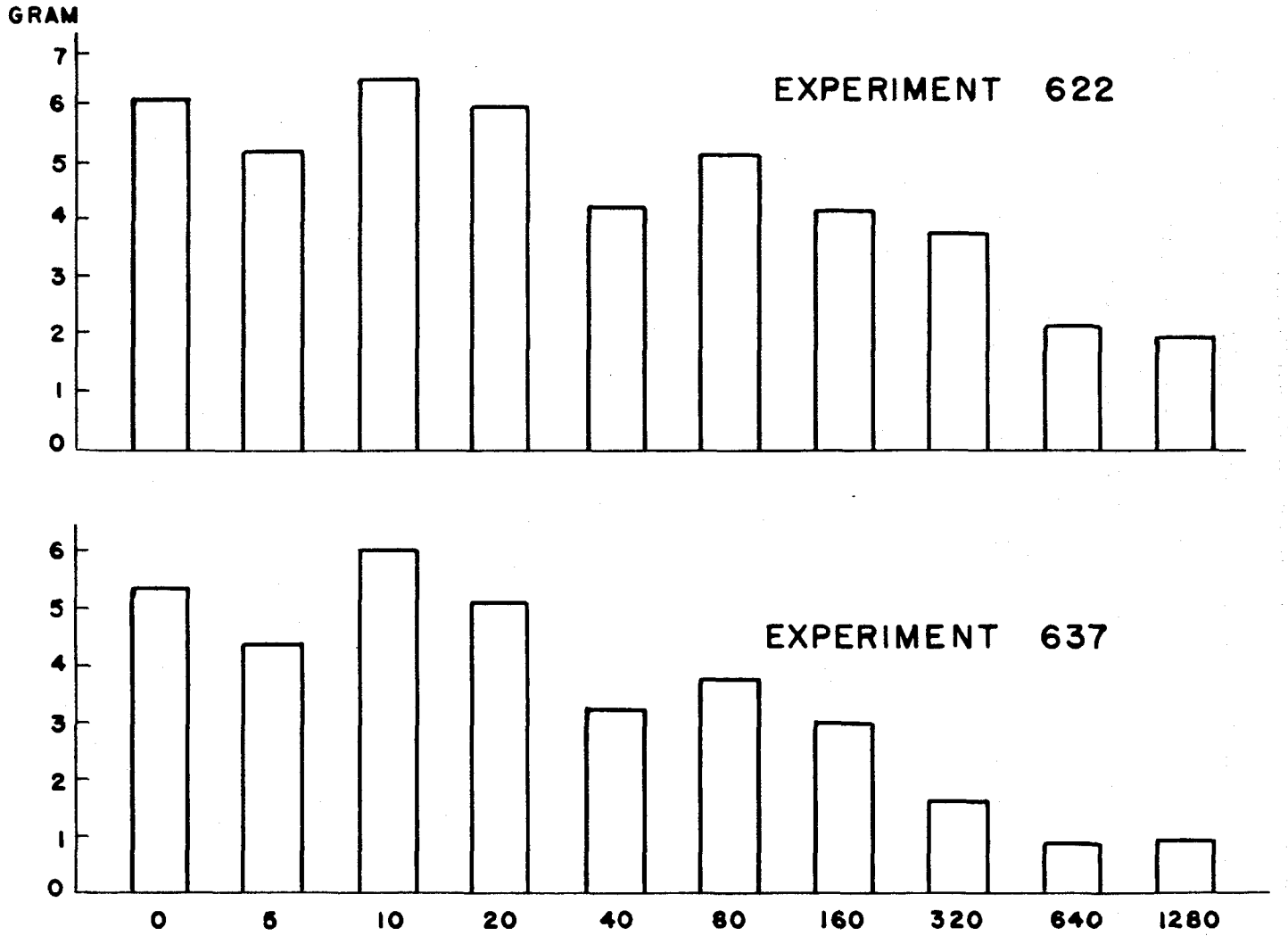
Stilbestrol mcg./pound basal ration	Experiment 622[c]		Experiment 637[c]	
	Ovaries	Follicles[d]	Ovaries	Follicles[d]
	(grams)	(number)	(grams)	(number)
0	6.20	10.0	5.70	12.5
5	5.35	7.0	4.35	7.5
10	6.55	8.0	6.00	9.3
20	6.00	4.5	4.60	12.5
40	5.37	4.5	3.20	3.0
80	5.25	5.5	3.84	6.7
160	4.15	2.0	2.49	0
320	3.75	0	1.63	0
640	3.17	1.5	0.80	0
1280	2.00	0	0.94	0
Average	4.18	4.3	3.55	5.1

[a,b]Explained in Table 6.

[c]Linear component significant at P = .01.

[d]Only follicles of 3 mm. or more considered.

Figure 21. Relationship of the level of stilbestrol to the weight of the ovaries



AVERAGE WEIGHT OF OVARIES

The effect of stilbestrol on the ovaries - Swine Experiment 637

Figure 22. Ovaries from Gilt 521 fed the basal ration - weight 4.9 grams and with 16 follicles of 3 mm. diameter or larger

Figure 23. Ovaries from Gilt 523 fed 10 mcg. stilbestrol per pound of ration - weight 5.71 grams and with 12 follicles of 3 mm. diameter or larger

Figure 24. Ovaries from Gilt 577 fed 1280 mcg. stilbestrol per pound of ration - weight 0.75 gram and with no follicles

Figure 25. Ovaries from Gilt 535 fed 1280 mcg. stilbestrol per pound of ration - weight 0.68 gram and with no follicles

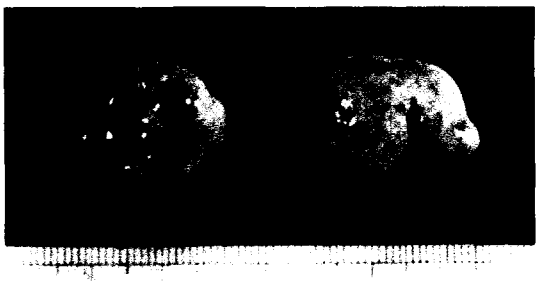


FIGURE 22

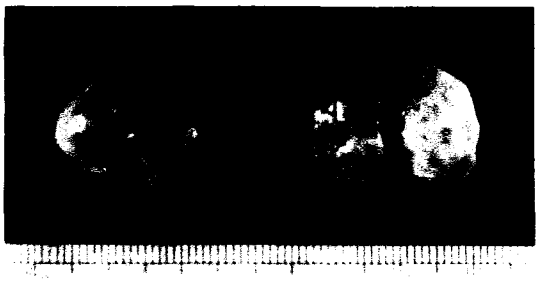


FIGURE 23

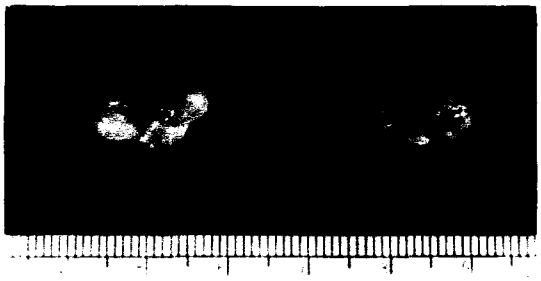


FIGURE 24

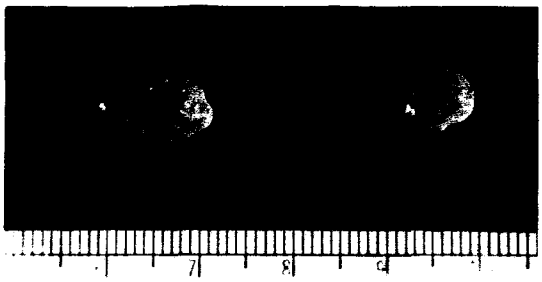


FIGURE 25

Liver weights were taken in both experiments for all gilts slaughtered and are shown in Table 9. The differences that would seem to be related to treatment are not significant statistically and show no similarity between experiments. These data suggest that one must be very careful in making inferences about the effect of stilbestrol on pork liver weights unless a large number of animals are involved. The treatment producing the most rapid gain in Experiment

Table 9. Level of stilbestrol and the weight of the liver - Swine Experiments 622[a] and 637[b]

Stilbestrol mcg./pound basal ration	Experiment 622[c]	Experiment 637[c]
	(grams)	
0	1533.5	1664.0
5	1631.0	1206.0
10	1513.5	1270.0
20	1384.5	1224.0
40	1529.5	1198.7
80	1440.0	1335.7
160	1725.5	1316.0
320	1627.0	1424.3
640	1668.5	1626.0
1280	1505.0	1539.7
Average	1555.8	1380.4

[a,b]Explained in Table 6.

[c]Treatment effect not significant at P = .05 or less.

622 contained gilts with an average liver weight of over 200 grams above the control gilts, but in Experiment 637 the average liver weight of gilts from the two pens making the greatest daily gains was over 300 grams less than the weight of the livers from the gilts that had not received stilbestrol.

The measurement of the pelvic inlet was taken in the hope that it might reveal any skeletal changes brought about by treatment. The transverse diameter showed a treatment effect and these data are presented in Table 10. These differences, although small were significant in Experiment 622, but not in Experiment 637. The control pigs in Experiment 622 gained at a slightly slower rate than the average of all pigs in the experiment, whereas the control pigs in Experiment 637 gained at the same rate as the average of all pigs in the trial and this difference in rate of growth may be the difference reflected.

Estrogenic activity of certain edible tissues. The data for the mouse assay of estrogenic activity for fat and for lean are presented in Table 11. The means of the uterine weights from mice fed lean tissue from pigs fed stilbestrol is either smaller than or almost identical with that response from the mice fed lean tissue from the control (zero level) pigs. Thus these data were not analyzed statistically. The

Table 10. Level of stilbestrol and the width of the pelvic inlet - Swine Experiments 622[a] and 637[b]

Stilbestrol mcg./pound basal ration	Experiment 622[c]	Experiment 637[d]
	(millimeters)	
0	6.7	6.4
5	6.6	7.0
10	6.5	6.7
20	7.0	6.8
40	7.0	6.5
80	7.5	6.3
160	7.4	6.6
320	7.8	6.5
640	7.2	7.1
1280	7.6	6.7
Average	7.13	6.66

[a,b]Explained in Table 6.

[c]Linear component significant at P = .05.

[d]Treatment effect not significant at P = .05 or less.

conclusion is that there was no evidence of estrogenic activity in the lean tissue assayed from pigs fed 10, 160, or 1280 mcg. of stilbestrol per pound of ration up to the time of slaughter of the pigs.

The data for the fat assay were not quite so clear-cut; hence these data for the 160 and 1280 mcg. levels of stilbestrol were analyzed by the method previously described.

Table 11. Uterine weights in pork lean and pork fat assay - Swine Experiment 637[a]

Tissue assayed	Stilbestrol mcg./pound basal ration	Mcg. of stilbestrol added per gram of basal diet				
		0	.005	.010	.020	Mean
		(milligrams)[b]				
Lean	0	9.4	10.9	15.4(9)	21.1(9)	14.2
	10	7.8	8.7	13.3	20.6	12.6
	160	10.7	10.5	14.6	22.5	14.6
	1280	9.0	10.2	14.6	22.5	11.8
Fat	0	5.64(8)	9.98	21.7	38.3	18.9
	10	5.58	9.18	26.3	34.2	18.8
	160	5.27	8.50	27.8	41.9	20.8[c]
	1280	5.16	8.92	19.7	52.6	21.8[c]

[a]Pork fat and lean from gilts left on the experimental rations up to time of slaughter.

[b]Each figure, excluding averages, represents an average of two pens of five mice each, except as figures in parentheses indicate a lesser number due to loss by death or wrong sex.

[c]Not significant at P = .05 or less

The treatment effects were not statistically significant.

Table 12 gives the data for the liver assay and Figures 26 to 30 show the liver assay curves for the standard and the unknown of the 160 and 1280 mcg. levels of stilbestrol feeding. Estrogenic activity was present in significant amounts at both no time off feed, and 24 hours off feed that contained stilbestrol, for the level of 1280 mcg. per pound of swine diet. A smaller amount of activity, also

Table 12. Uterine weights in pork liver assay - Swine Experiment 637[a]

Hours exp. ration withdrawn before slaughter	Stilbestrol mcg./pound basal ration	Mcg. of stilbestrol added per gram of mouse diet				Estrogenic activity equivalent to mcg. Stilbestrol per gram of:	
		.005	.010	.020	Mean	Dried liver	Fresh liver
		(milligrams)					
0	0	9.1	12.5	21.3	14.3	---	---
	10	8.5	10.4	23.8	14.2	b	b
	160	15.2	24.5	32.1	23.9	0.02986[c]	4.15[c]
	1280	50.6	59.1	60.1	56.6	0.15440[c]	21.46[c]
24	0	6.6	11.7	27.4	15.2	---	---
	10	7.8	11.8	22.6	14.1	b	b
	160	9.4	11.8	30.1	17.1	0.009069[d]	1.25[d]
	1280	47.4	45.5	53.9	48.9	0.088870[c]	12.34[c]
48	0	12.9	15.9	26.7	18.5	---	---
	10	11.2	10.1	19.4	13.6	b	b
	160	10.8	14.8	30.7	18.8	b	b
	1280	13.5	17.8	29.5	20.3	0.00241[d]	0.3346[d]

[a]Liver from gilts fed four levels of stilbestrol and with stilbestrol withdrawn from pig rations at three time intervals before slaughter.

[b]Not analyzed statistically.

[c]Amount of estrogenic activity significant at P = .05.

[d]Amount of estrogenic activity not significant at P = .05 or less.

Figure 26. Uterine weights in pork liver assay plotted, for standard and unknown, against stilbestrol or stilbestrol plus estrogenic activity per gram of mouse diet - unknown from a pig fed 160 mcg. stilbestrol per pound of ration and not withheld from feed before slaughter

Curves fitted from the equations:

$y_s = 4.45 + 945.9x$, and

$y_u = 13.6 + 949.9x$, with the mean response of each pen of mice plotted for comparison

M = 0.011946, and is significant at P = .05

The value of M represents mcg. stilbestrol or equivalent estrogenic activity per gram of mouse diet and is the horizontal distance between the standard and unknown response curves

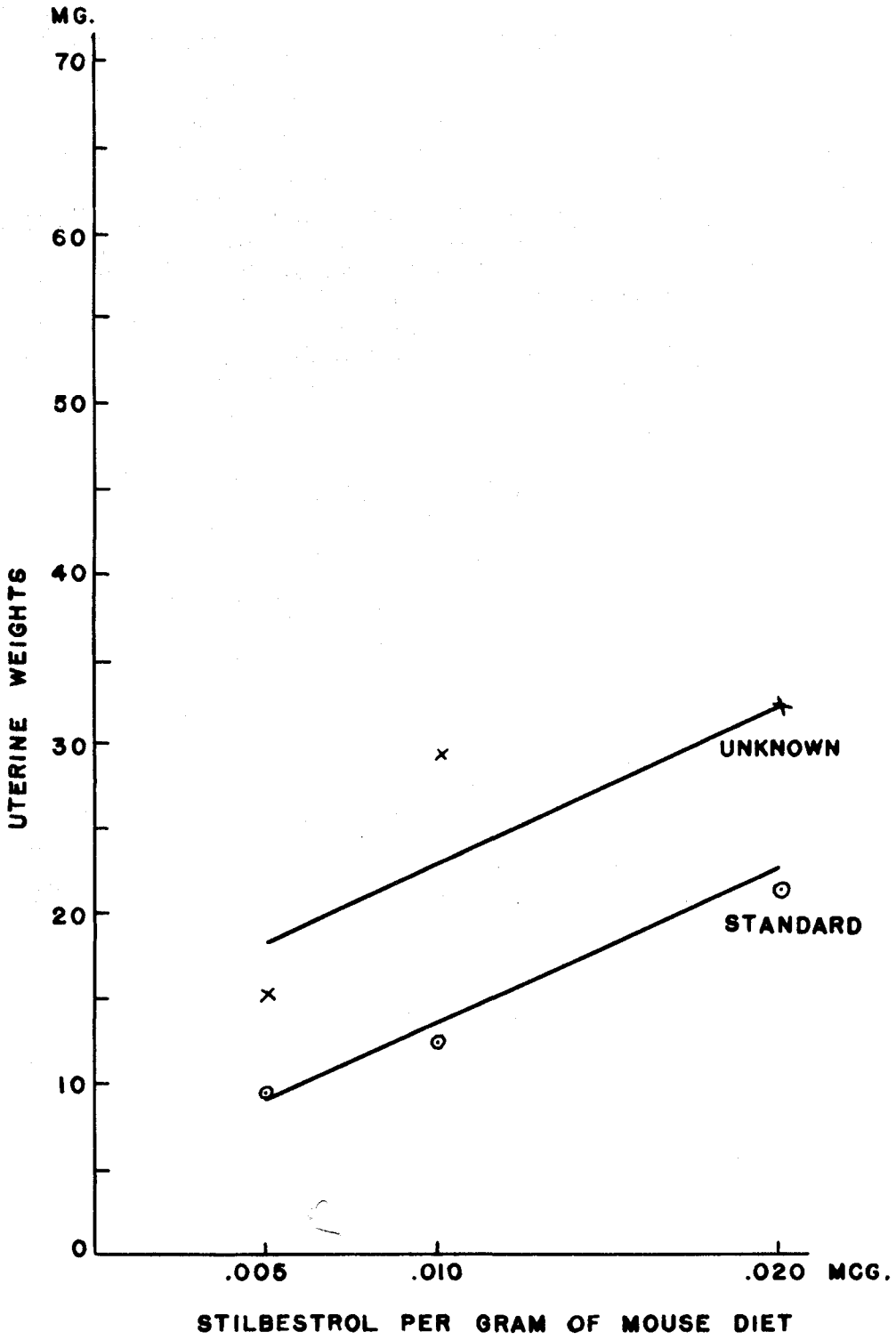

MG.
70
60
50
40
30
20
10
0
UTERINE WEIGHTS
UNKNOWN
STANDARD
.005
.010
.020 MCG.
STILBESTROL PER GRAM OF MOUSE DIET

Figure 27. Uterine weights in pork liver assay plotted, for standard and unknown, against stilbestrol or stilbestrol plus estrogenic activity per gram of mouse diet - unknown from a pig fed 160 mcg. stilbestrol per pound of ration and not withheld from feed before slaughter

Curve fitted from the equations:

$y_s = -1.67 + 1428.63x$, and

$y_u = -0.25 + 1428.63x$, with only one curve drawn as

$M = 0.00096408$, and is not significant at $P = .05$

The mean response of each pen of mice is plotted for comparison

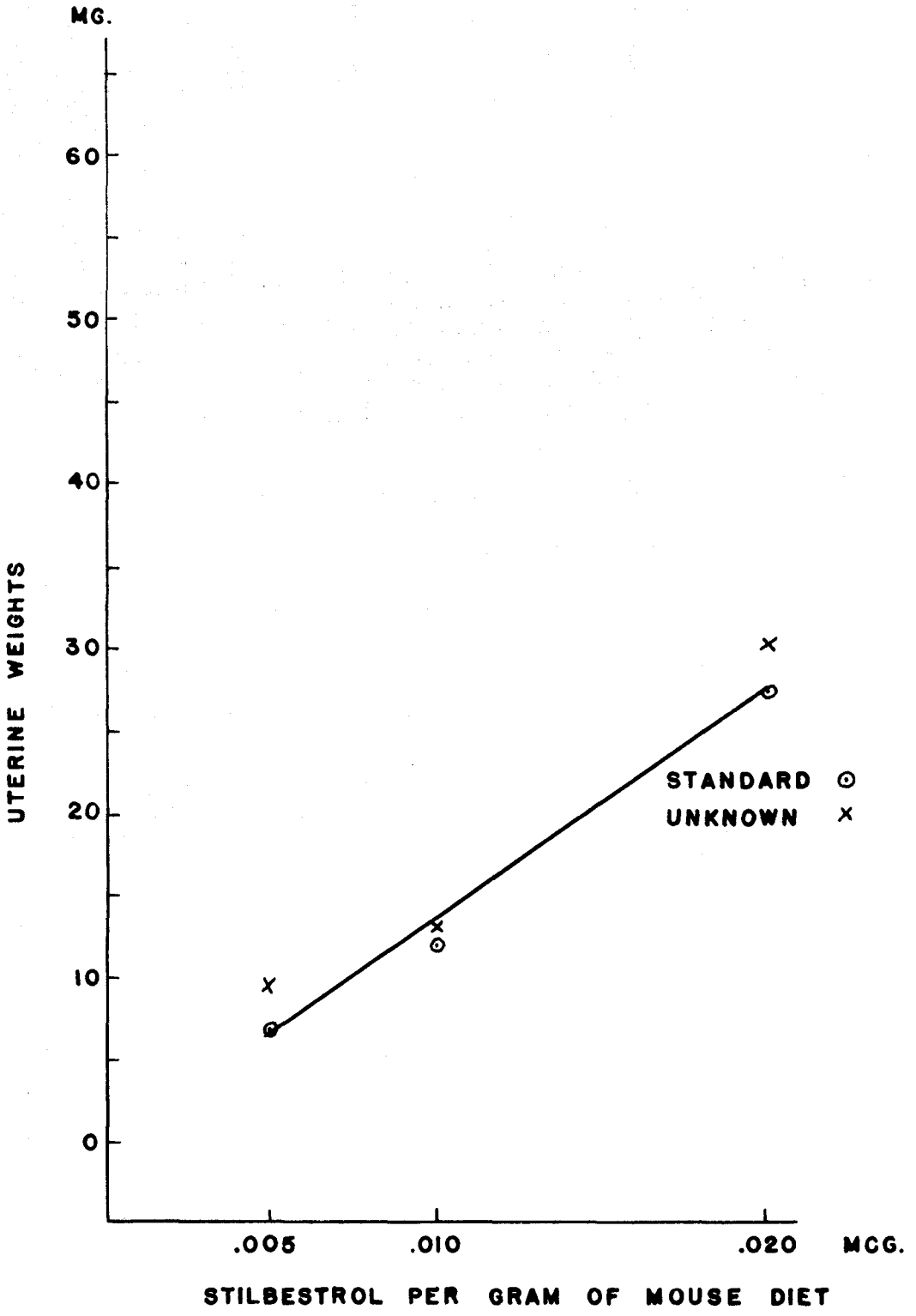

MG.
60
50
40
30
20
10
0
UTERINE WEIGHTS
STANDARD
UNKNOWN
.005
.010
.020
MCG.
STILBESTROL PER GRAM OF MOUSE DIET

Figure 28. Uterine weights in pork liver assay plotted, for standard and unknown, against stilbestrol or stilbestrol plus estrogenic activity per gram of mouse diet - unknown from a pig fed 1280 mcg. stilbestrol per pound of ration and not withheld from feed before slaughter

Curves fitted from the equations:

$y_s = 4.45 + 683.76x$, and

$y_u = 49.9 + 683.76x$, with the mean response of each pen of mice plotted for comparison

M = 0.06041824, and is significant at P = .05

The value of M represents mcg. stilbestrol or equivalent estrogenic activity per gram of mouse diet and should be the horizontal distance between the standard and unknown response curves, but in this case the unknown is beyond the range of the assay and the value of M cannot be checked graphically

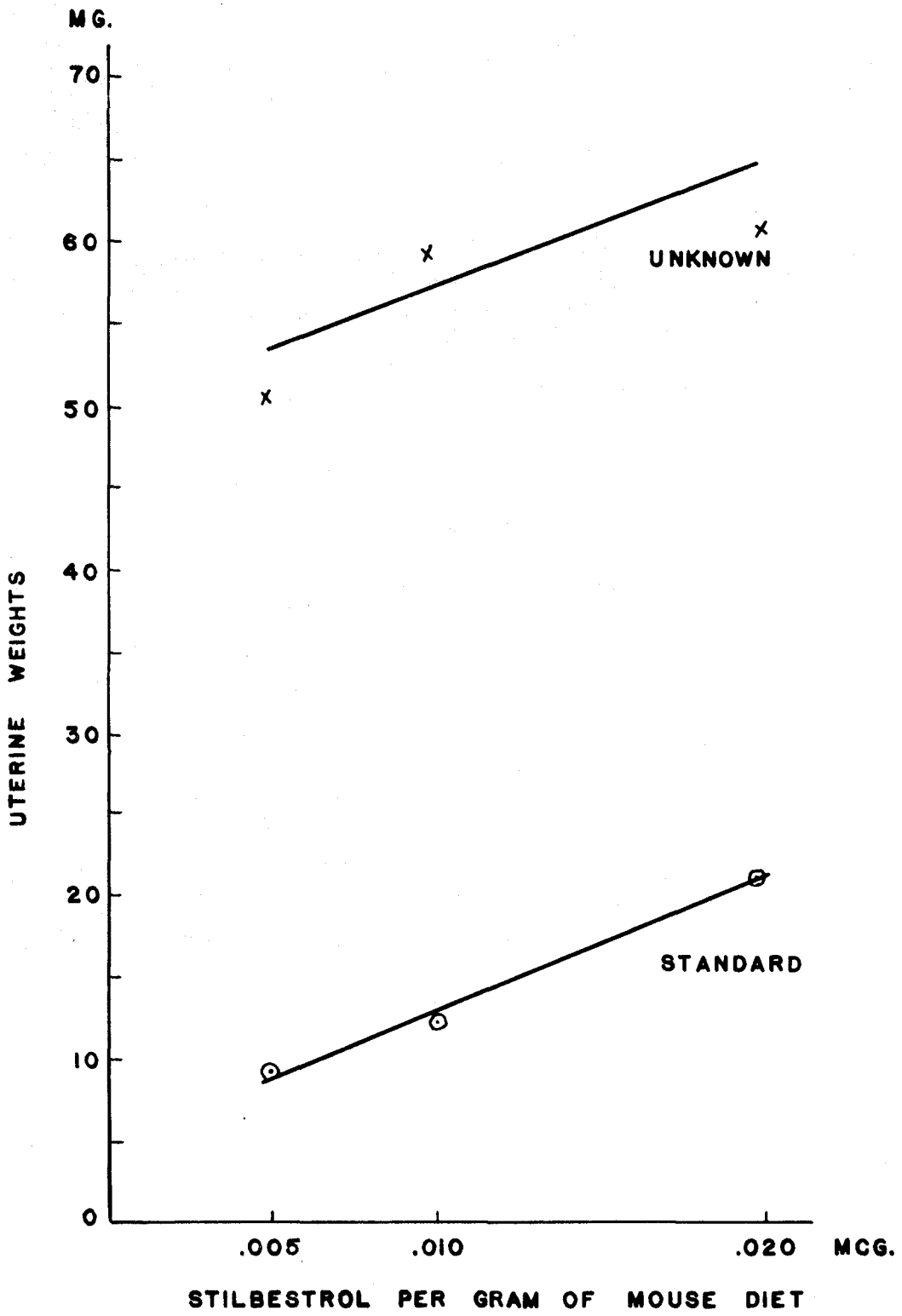

MG.
70
60
50
40
30
20
10
0
UTERINE WEIGHTS
UNKNOWN
STANDARD
.005
.010
.020
MCG.
STILBESTROL PER GRAM OF MOUSE DIET

Figure 29. Uterine weights in pork liver assay plotted, for standard and unknown, against stilbestrol or stilbestrol plus estrogenic activity per gram of mouse diet - unknown from a pig fed 1280 mcg. stilbestrol per pound of ration and withheld from feed 24 hours before slaughter

Curves fitted from the equations:

$y_s = 7.00 + 948.72x$, and

$y_u = 40.11 + 948.72x$, with the mean response of each pen of mice plotted for comparison

M = 0.03555, and is significant at P = .05

The value of M represents mcg. stilbestrol or equivalent estrogenic activity per gram of mouse diet and should be the horizontal distance between the standard and unknown response curves, but in this case the unknown is beyond the range of the assay and the value of M cannot be checked graphically

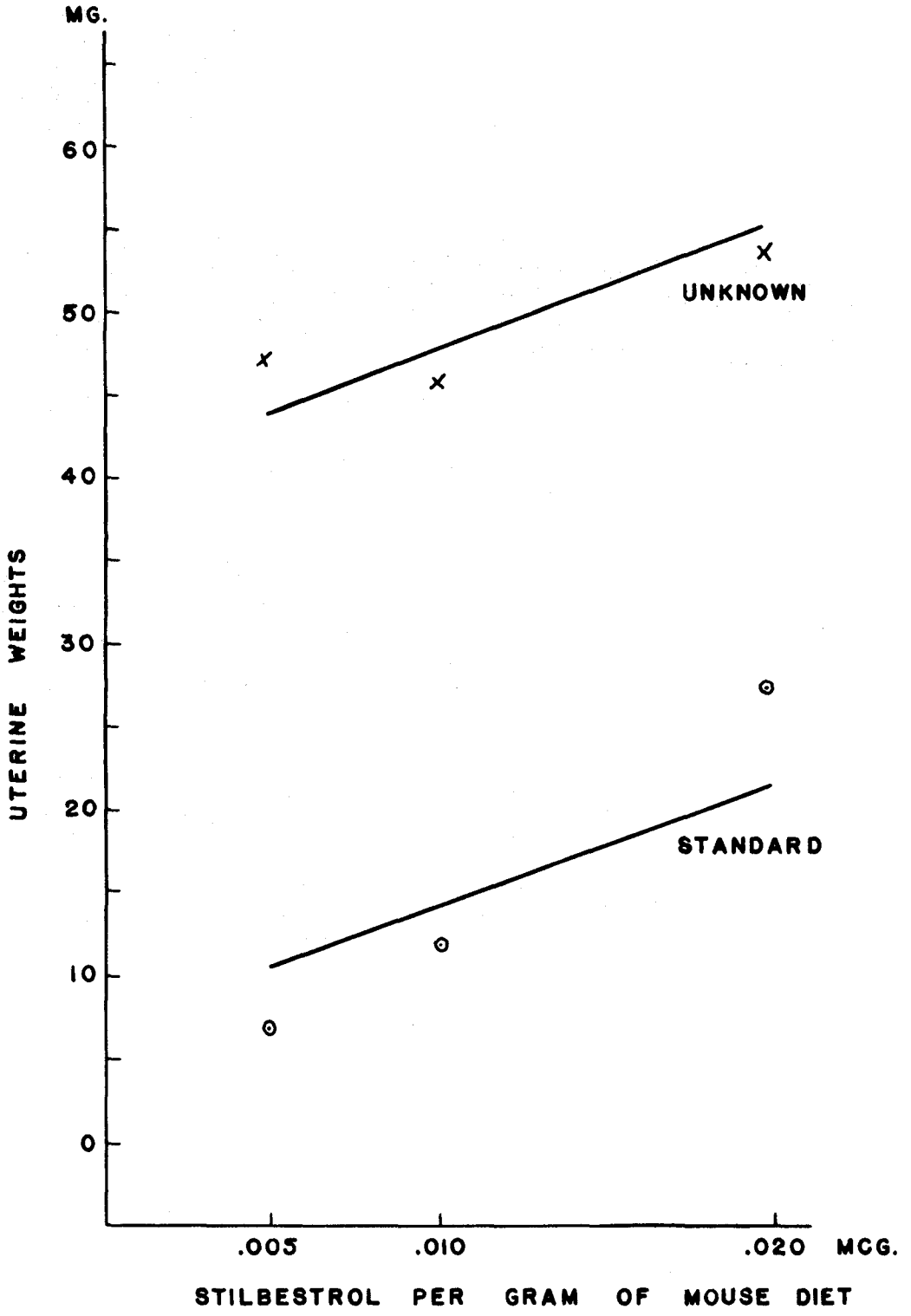

MG.
60
50
40
30
20
10
0
UTERINE WEIGHTS
UNKNOWN
STANDARD
.005
.010
.020
MCG.
STILBESTROL PER GRAM OF MOUSE DIET

Figure 30. Uterine weights in pork liver assay plotted, for standard and unknown, against stilbestrol or stilbestrol plus estrogenic activity per gram of mouse diet - unknown from a pig fed 1280 mcg. stilbestrol per pound of ration and withheld from feed 48 hours before slaughter

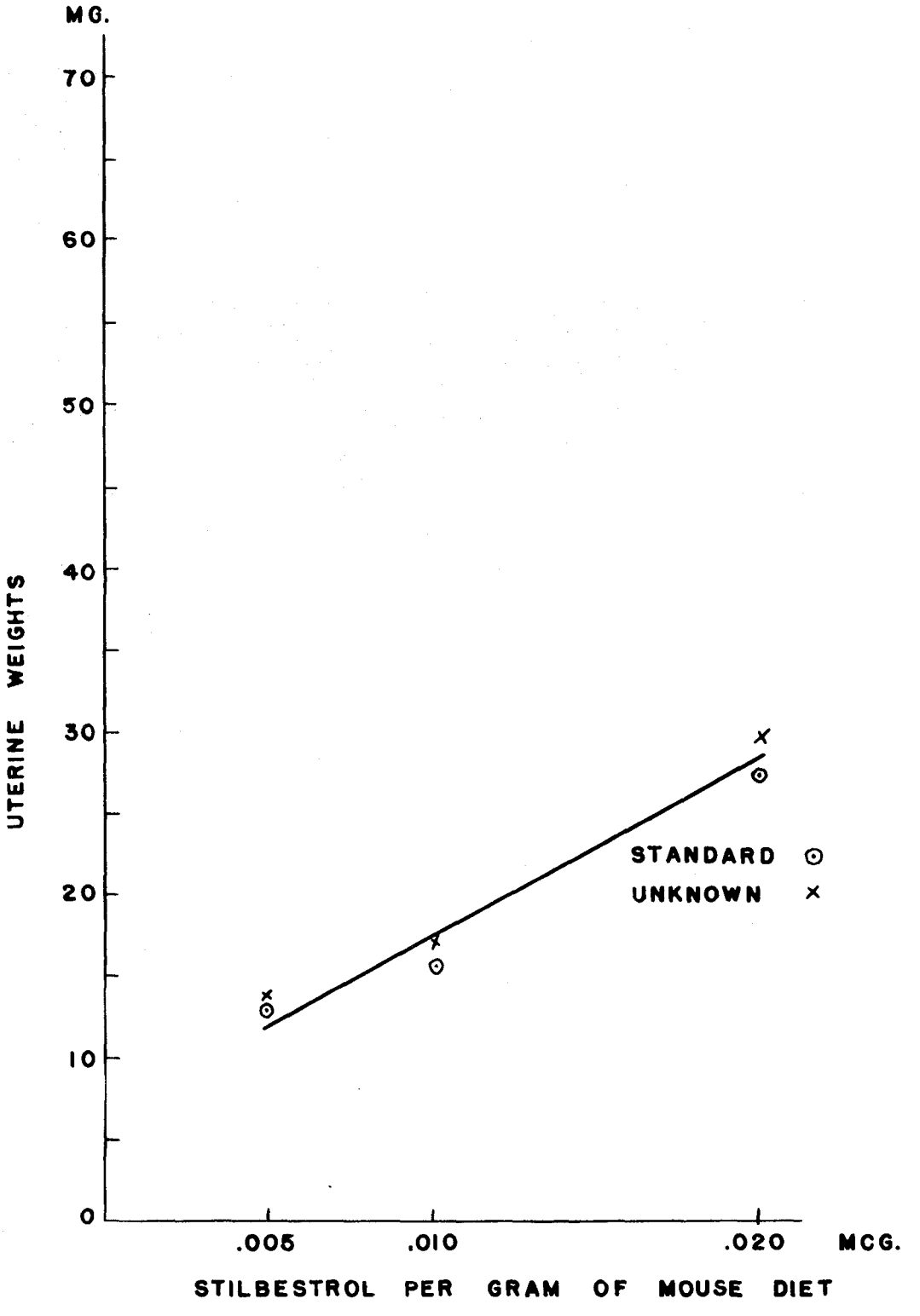
Curves fitted from the equations:

$y_s = 6.44 + 1068.37x$, and

$y_u = 6.85 + 1068.37x$, with only one curve drawn as

$M = 0.00096408$, and is not significant at $P = .05$

The mean response of each pen of mice is plotted for comparison


MG.
70
60
50
40
30
20
10
0
UTERINE WEIGHTS
STANDARD ⊙
UNKNOWN ×
.005
.010
.020
MCG.
STILBESTROL PER GRAM OF MOUSE DIET

significant, was found for the 160 mcg. per pound level when the pigs were not taken off the experimental ration before slaughter. In all other cases the amount of estrogenic activity was too small to be statistically significant or the data indicate that no statistical analysis was needed for interpretation.

No evidence of estrogenic activity was detected by this assay from liver tissue from pigs fed 10 mcg. of stilbestrol per pound of feed regardless of whether the feed containing stilbestrol was removed 0, 24, or 48 hours before slaughter. Liver from pigs fed 160 mcg. of stilbestrol per pound of feed showed nearly three times the estrogenic activity (0.03 mcg. per gram of dry liver) as that reported by Stob et al. (75) for beef muscle, and one and one-half times the amount these workers report for dried lamb tissue, all from animals that had been implanted with pellets of stilbestrol. In the present experiment this activity had all disappeared after 24 hours off feed.

The higher level of 1280 mcg. of stilbestrol per pound of swine ration produced the equivalent response of 21.46 mcg. of stilbestrol per pound of fresh liver for no time off feed, which is slightly less than that reported by Whiting et al. (84) for the kidney fat of stilbestrol implanted lambs - equivalent to no time off feed. Within 24 hours

after the last feed containing stilbestrol, 57.5 percent of the original activity remained but by the 48th hour, after the last feed containing stilbestrol, none or a statistically insignificant amount was shown by the mouse assay technique employed.

In all assays analyzed statistically the linear regression of mean uterine weight on level of stilbestrol was significant at P = .01. In all cases the departure from parallelism was not significant at P = .05 or less.

Discussion

These two trials with growing-finishing pigs indicate that the feeding of stilbestrol at levels up to 1280 mcg. per pound of feed results in only small and insignificant improvement in rate of gain. These findings agree with those of Perry *et al.* (63) who report that the feeding of 2.5 mg. of stilbestrol per pig per day from weaning to 125 pounds, then 5 mg. per head daily to market weight was without effect, and with those of Beeson *et al.* (7) who found a level of 2 mg. of stilbestrol per pig daily to be without effect on rate of gain. Both groups of workers report clear cut mammary gland stimulation and swelling of the vulva at these rates that, as used initially, would fall

between our 80 and 160 mcg. per pound of feed levels.

On the other hand, all levels used in this investigation are well below the 50 mg. of stilbestrol per pig per day fed by Braude (15) who reports slight improvement in rate of gain and feed efficiency for castrated male pigs. Braude (16) found that it was necessary to feed iodinated casein, a thyroid stimulant, with stilbestrol to produce weight gain and feed efficiency increases of significant magnitude. This suggests that stilbestrol alone does not raise the metabolic rate in pigs, and the findings in this trial, as well as those of Beeson et al. (7), that the trend was for an equally lean carcass from the feeding of stilbestrol, indicates that stilbestrol does not lower the basal metabolism in the pig. That the levels fed by Braude (16, 17) or those of Barber et al. (6) are near the upper limit of tolerance for the pig is suggested by the work of Taylor and Gordon (79) who report five cases of toxicity from the feeding of 6 mg. of stilbestrol per pound of feed, with thyroxine, to growing-fattening pigs. The present work indicates that the level of 160 mcg. of stilbestrol per pound of feed is the highest practical level for gilts as excessive swelling of the vulva becomes too objectionable at higher levels. Thus, the opportunity, if any, for stilbestrol in swine rations would seem to be limited to barrows,

if higher levels are needed, or to the inclusion of another compound or compounds that will act antagonistically to stilbestrol in its effect on the vulva and teats and yet synergestically to it in the matter of rate of gain and efficiency of feed conversion. Assuming that the above is possible, there are other considerations. Estrogenic activity was present in the liver of gilts fed either 160 or 1280 mcg. of stilbestrol per pound of ration when the pigs were fed this ration up to the time of slaughter. At the lower level, this activity was not detectable for pigs taken off feed 24 hours before slaughter, but at the 1280 mcg. level 48 hours of time off feed was necessary before the liver assay proved negative. This suggests that stilbestrol would be suitable only for inclusion in a complete ration rather than in a supplement to be offered free-choice to pigs, as excessive consumption of the supplement would confound any recommendations as to minimum time of withdrawal of feed containing stilbestrol before slaughter of the pigs.

The finding that the increase in the size of the reproductive tract due to the feeding of stilbestrol varied with different lines of breeding is not surprising. Green *et al.* (41) found that the androgen excretion rate of boars of different lines of breeding was quite different and a similar difference may hold for the general sex hormone

balance. Stockard (76) concluded that breed characteristics in dogs are apparently due to endocrine peculiarities that are transmitted in Mendelian order.

The evidence of stimulation of the ovaries, vulva, and teats produced by the higher levels of stilbestrol without evidence of sexual excitement such as riding and ranting suggests that it is not the hormone level of estrus that is being duplicated. The other possibility could be an approximation of the hormone level of pregnancy. That this might be true is suggested by the relaxation of the cervix as uterine contractions are greatest during estrus and least during pregnancy, Turner (80). The indication that treatment caused a slight enlargement of the width of the pelvic inlet is also suggestive of this fact, but no explanation of the possible mode of action is apparent. The same general effect has been observed, with mice, by Hall and Newton (43) who found that the effect on the pelvic inlet was independent of the size of dose. On the other hand, Andrews et al. (3) found, with cattle, that stilbestrol caused an increase in rate of gain and relaxation in the loin region with an elevation in the tailhead when the compound was administered as a pellet. This would indicate that the size of dose is the factor determining whether an increase in sexual excitement results from the administration

of stilbestrol, yet suggests at the same time that the hormone balance of late pregnancy may be approached.

The drastic reduction in ovary size and in the number of follicles resulting from the feeding of 320 mcg. of stilbestrol or more per pound of ration is accompanied by an increase in vulva size that is also dependent upon the level of feeding. Further work with the level of stilbestrol necessary to cause temporary damage to the ovary might be correlated with the observed and measured enlargement of the vulva.

Summary

Levels of stilbestrol of 0, 5, 10, 20, 40, 80, 160, 320, 640, and 1280 mcg. per pound of ration were fed in two experiments to a total of 140 growing-finishing pigs. Fortified corn-soybean oil meal rations were fed in concrete pens to pigs from an initial weight of approximately 35 pounds to a termination weight of 200 pounds.

The feeding of stilbestrol under the conditions of these experiments had no significant effect on rate of gain or feed efficiency. In Experiment 622 the lower levels of either 5 or 10 mcg. of stilbestrol per pound of ration gave a small stimulation in rate of gain, and pigs on the

160 mcg. per pound level gained 1.86 pounds per day as compared to 1.67 pounds per day for the control pigs. In Experiment 637 pigs on the 80 and 160 mcg. per pound of ration levels made the most rapid gains and were the most efficient in feed conversion, but the differences were less than in the first experiment. Differences in live probe measurements, dressing percentages, and in carcass shrink were small and not statistically significant.

The gilts fed 320 mcg. or more of stilbestrol per pound of ration developed gross enlargement of the vulva by the third day of the trial, with the degree of enlargement varying with the level of stilbestrol fed. The gilts fed 160 mcg. of stilbestrol per pound of ration developed slight enlargement of the vulva by the eighth day of the experiment, but this condition was not observed in the gilts on any lower level at any time during the trial. Enlargement of the teats, in gilts, and of the rudimentary teats in barrows was evident by the 30th day of each experiment at those levels that stimulated the size of the vulva.

The feeding of stilbestrol at levels of from 5 to 1280 mcg. per pound of ration produced a significant increase in the diameter of the cervix and a significant increase in the weight of the reproductive tract. The 10 mcg. of stilbestrol per pound of feed level increased the size of the

ovary in both experiments, whereas all other levels of stilbestrol decreased ovary size as well as follicle diameter and development.

Estrogenic activity was present in the liver of a gilt fed 1280 mcg. of stilbestrol per pound of feed up to the time of slaughter and a smaller amount was detected in another gilt fed the same ration to within 24 hours of the time of slaughter. A smaller estrogenic response was detected in the liver from a gilt fed 160 mcg. per pound of feed up to the time of slaughter. Other assay comparisons showed that all estrogenic activity had disappeared from the liver representing the 1280 mcg. level by 48 hours after the last feed containing stilbestrol, and from the liver from the 160 mcg. per pound level of feeding by 24 hours after the removal of the experimental ration. No activity could be detected in the livers from the gilt fed 10 mcg. of stilbestrol per pound of feed up to the time of slaughter. The liver from the gilt fed the highest level of stilbestrol, and not withheld from this ration before slaughter, contained estrogenic activity equivalent to 21.46 mcg. of stilbestrol per pound of fresh liver. Within 24 hours after the last feed of the stilbestrol ration, 42.5 percent of this activity had disappeared and no activity was detected after 48 hours from the time of removal of the experimental ration.

Part II. Studies with Lambs

Lamb Experiment 1

Method and materials. To study the effects of feeding methyl testosterone at two different levels and that of testosterone propionate administered by subcutaneous injection upon ewe lambs, a randomized block design was used with four treatments and four lambs per treatment. Allotment was made from outcome groups by weight. The lambs were cross-bred ewe lambs of Western origin and averaged 72 pounds initially in full fleece. The lambs were vaccinated for enterotoxemia, ear-tagged, and paint-stamped during a ten-day pre-experimental period. The lambs were fed twice daily, for a three-hour period, from individual self feeders and were penned in an adjacent pen when not eating. Both the feeding stalls and the pens were under cover. Salt, in block form, and water were available in the group pen. Two-day weights were taken at the beginning, 14-day weights for the duration, and two-day weights at the close of the trial. The trial period was from December 18, 1953 to March 12, 1954 - a period of 84 days.

The total mixed ration used contained the following ingredients, in percent: ground alfalfa hay, 50; cane

molasses, 10; cracked corn, 34.5; and soybean oil meal, 5.5. The control lambs and those receiving testosterone propionate by injection received soybean oil meal to which had been added the same amount of corn oil per unit of meal as was necessarily used as a carrier for the premix fed the lambs receiving methyl testosterone. The methyl testosterone was dissolved in corn oil, mixed by hand with a small amount of soybean oil meal, then with a larger amount of soybean oil meal by a mechanical mixer. Methyl testosterone was added to the supplement in such amounts that the daily intake per lamb, for the two treatments, would be 8.5 and 42.5 mg. per head daily, with an average daily feed intake of 3.5 pounds of total ration. The testosterone propionate was partially dissolved in sesame oil and injected subcutaneously in the neck region each 14th day at the rate of 3.3 mg. per 100 pounds of body weight per day.

At the conclusion of the trial all lambs were shorn and then slaughtered through the Iowa State College meats laboratory with a record made of the weights of the following: pelt, warm carcass, chilled carcass, liver, thyroid, adrenals, ovaries, and uteri. The 9, 10, and 11th rib cut was weighed, divided into separable fat, lean, and bone and moisture and fat determinations were made. A blood hemoglobin determination was made on the 81st day of the

trial.

The several groups of data were analyzed statistically and the results of the analyses are included in the Appendix, Tables 42 and 43.

Results and discussion. The data for weight gains and feed are shown in Table 13, and are presented graphically in Figure 31. Testosterone propionate injected at the rate of

Table 13. Growth and fattening stimulation in ewe lambs with methyl testosterone and testosterone propionate - Lamb Experiment 1[a]

Group	Additions to the basal ration	Daily gain[b]	Feed per day	Feed per 100 lb. gain
			(pounds)	
1	None	0.38	3.22	847.33
2	8.52 mg. methyl testosterone	0.44	3.51	800.00
3	42.86 mg. methyl testosterone	0.44	3.53	816.64
4	Testosterone propionate implant[c]	0.51	3.61	710.23
	Average	0.44	3.47	793.57
	L.S.D. at .05	0.057	--	--

[a]Average of four lambs per group.

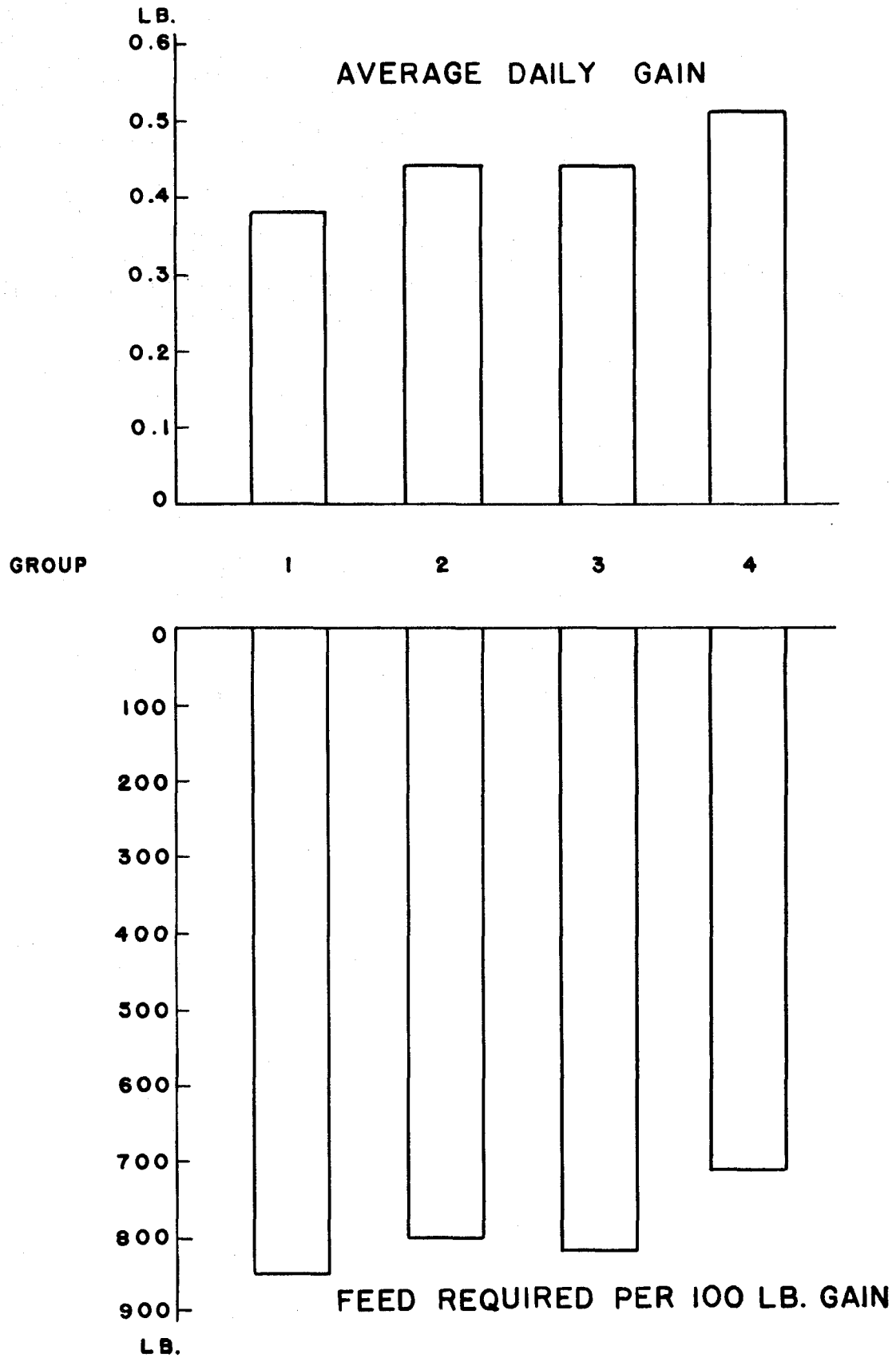
[b]Treatment effects significant at P = .05 or less.

[c]The methyl testosterone and testosterone propionate used in this and the three subsequent lamb trials were furnished by Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

Figure 31. Treatment effect on average daily gain and feed required per 100 pounds gain

Groups:

1. Basal ration
2. Basal ration plus 8.5 mg. methyl testosterone per lamb per day
3. Basal ration plus 42.5 mg. methyl testosterone per lamb per day
4. Basal ration plus testosterone propionate injected at the rate of 3.3 mg. per 100 lb. body weight per day


LB.
0.6
0.5
0.4
0.3
0.2
0.1
0
AVERAGE DAILY GAIN
GROUP
1
2
3
4
0
100
200
300
400
500
600
700
800
900
LB.
FEED REQUIRED PER 100 LB. GAIN

3.3 mg. per 100 pounds of body weight per day increased gains by 38 percent over the control lambs and this difference was highly significant. These results agree generally with those of O'Mary et al. (61), and of Means et al. (56), who report some stimulation in weight gains in lambs from the implantation of a 15 mg. or 30 mg. pellet of testosterone propionate, and of Burris et al. (21) with beef heifers.

Methyl testosterone fed at levels of either 8.5 or 42.5 mg. per lamb daily increased gains by approximately 16 percent and this difference is significantly greater than the gains made by the control lambs.

Average daily feed intake was increased, though not significantly, and the feed required per 100 pounds of gain was reduced by treatment, but not by a significant amount.

The data for carcass characteristics are shown in Table 14. Carcass grades were lowered significantly, over a full grade, by treatment with testosterone propionate and dressing percent was reduced slightly as compared to the control lambs. The lambs that had been treated with testosterone propionate went to the killing floor 4.75 pounds heavier than the group fed the higher level of methyl testosterone, but yielded a warm carcass of almost identical weight, and a chilled carcass of .75 pound less weight. An average pelt weight of 2.15 pounds more for

Table 14. The effect of methyl testosterone and testosterone propionate on shrink in killing and in chilling, and on dressing percentage and carcass grade - Lamb Experiment 1[a]

Group	Treatment[b]	Slaughter weight	Pelt weight	Warm carcass weight	Chilled carcass weight	Shrink in chilling	Dressing percentage[c]	Carcass grade[d,e]
			(pounds)			(percent)		
1	Control	91.25	8.12	46.50	45.12	2.97	49.45	7.25
2	Methyl testosterone	96.25	8.75	48.10	47.31	1.64	49.15	7.00
3	Methyl testosterone	96.25	8.06	49.81	49.00	1.63	50.91	7.75
4	Testosterone propionate	101.00	10.19	49.85	48.21	3.29	47.74	4.50
	Average	96.19	8.78	48.56	47.41	2.38	49.31	6.62

[a,b]Explained in Table 13.

[c]Significant at P = .05 or less. L.S.D. 1.00.

[d]Lambs were shorn immediately before slaughter. Basis shorn liveweight.

[e]Carcass grades computed using the following numerical system: choice, 8; choice minus, 7; good plus, 6; good, 5; good minus, 4; utility plus, 3; and utility, 2.

the lambs implanted with testosterone propionate is only a part of this greater loss in killing, and greater shrink in chilling - 3.25 percent as compared to 1.63 percent - accounts for the lower chilled weight. It was observed from the killing floor that the lambs that had been implanted with testosterone propionate had an unusually large amount of intestinal fat and this would lower carcass yield. Lambs implanted with testosterone propionate yielded growthy, long necked carcasses that were lacking in finish and in fullness of loin as compared to those of the other groups. Somewhat similarly, O'Mary et al. (62) found that ewe lambs that had been implanted with a 15 mg. pellet of testosterone propionate yielded carcasses showing less fat than the control carcasses in the trial.

The carcasses of the first three groups of lambs were quite similar, with the lambs fed the 42.5 mg. level of methyl testosterone yielding the highest grading carcasses.

The data for the comparison of the 9, 10, and 11th rib cut are shown in Table 15. As would be expected, the carcasses that graded highest yielded rib cuts with more fat and less water than did the lower grading carcasses. The differences in the percent of bone and percent of lean are small and it may be that these differences are mere reflections of the differences in percentage of fat in the

Table 15. A comparison of the composition of the 9, 10, and 11th rib cut - Lamb Experiment 1[a]

Group	Treatment[b]	Weight of cut[c]	Bone	Lean	Water	Fat
				(percent)		
1	Control	5.67	13.15	25.17	26.94	37.64
2	Methyl testosterone	5.60	13.52	24.65	27.82	36.32
3	Methyl testosterone	5.89	12.71	24.45	23.82	40.44
4	Testosterone propionate	5.72	13.40	25.19	27.37	36.21
	Average	5.72	13.19	24.86	26.49	37.65

[a,b]Explained in Table 13.

[c]Refers to percent of carcass represented by the rib cut.

rib cut, and in individual variation. It would seem that the response to the two levels of methyl testosterone was quite different even though both groups gained the same total weight while on feed. Those lambs fed the 42.5 mg. level of the drug contained 4 percent more fat and 4 percent less water in the rib cut than did carcasses from lambs that were fed the 8.5 mg. level of the same compound.

The various organ and tissue measurements are presented in Table 16. Although differences exist in the average

Table 16. The effect of methyl testosterone and testosterone propionate on the weight of various organs of the body - Lamb Experiment 1[a]

Group	Treatment[b]	Liver	Thyroid[c]	Adrenals	Uteri	Ovaries
				(grams)		
1	Control	713.79	3.72	2.62	18.61	1.17
2	Methyl testosterone	763.00	2.91	2.48	15.76	1.26
3	Methyl testosterone	822.50	3.48	2.50	17.23	1.42
4	Testosterone propionate	881.75	5.23	1.89	17.06	0.99
	Average	795.26	3.83	2.37	17.16	1.21
	L.S.D. at .05	--	0.47	--	--	--

[a,b]Explained in Table 15.

[c]Significant at P = .05.

weights of the liver, thyroid, adrenals, uteri, and ovaries, only those for the thyroids are significant. The feeding of 8.52 mg. of methyl testosterone daily significantly depressed thyroid weight, while the feeding of the same synthetic androgen at a level of 42.5 mg. daily had no effect on thyroid weight, and the injection of testosterone propionate significantly increased the weight of the thyroids. Histological examination of the thyroids of the

testosterone propionate group and of the controls revealed no apparent difference between the two. The results of this trial indicate that, using the control group as the standard, definite indications of added growth were produced by the injection of testosterone propionate in ewe lambs, and that greater fat deposition, without evidence of skeletal growth, occurred in those lambs fed the 42.5 mg. level of methyl testosterone. The scope of this study did not permit the determination as to whether the differences in thyroid weights in the various groups were accompanied by changes in the metabolic rate. Burris et al. (20) found that intramuscular injections of testosterone significantly increased thyroid weight in beef steers and heifers that had gained significantly more than the control calves by which the thyroid size was compared. They also showed that the secretory activity of the thyroid gland was increased by treatment and that the stores of thyroxine in the gland of the treated calves were decreased. Whereas the present observation could be interpreted to agree with Burris et al. (20) in one case, the significantly smaller thyroid weight for the lambs fed methyl testosterone at the level of 8.5 mg. per lamb daily and that gained significantly more than the controls, calls for a different explanation. The findings in this case may be more in line with those of

Rupp and Paschkis (70) who found that the nitrogen retention and weight producing action of testosterone was independent of the thyroid in the rat. These workers also found the same action to be independent of the pituitary, also in the rat. On the other hand McCullough and Rossmiller (55) report an increase in metabolic rate of 12 to 54 percent for humans from the clinical use of methyl testosterone, causing one to wonder if increased enzyme action alone could cause such an increase in metabolic rate without the involvement of the thyroid. The basal ration in this experiment was calculated to contain 11.5 percent protein, and this is higher than is usually recommended. This was done with the thought that any increase in the retention of nitorgen, through whatever mechanism accomplished, by androgens would be retarded unless the ration contained an abundance rather than a minimum of protein. It is unfortunate that the results of many of the trials reviewed did not give the protein content of the ration used.

The effect of testosterone propionate on adrenal gland weight is not significant, but is suggestive of an inhibitory action on the adrenals and perhaps this effect is mediated through inhibiting the action of the pituitary. This trend agrees with Sahyun (71) who describes this action as a general effect of testosterone.

Summary. Sixteen ewe lambs of Western origin and averaging 69 pounds in weight were allotted from outcome groups by weight to four groups for an 84-day trial conducted in the December to March period of 1953-1954. The lambs were individually fed an 11.5 percent protein basal ration of cracked corn, soybean oil meal, cane molasses, and ground alfalfa hay. Additions to the basal ration consisted of: (1) none; (2) 8.5 mg. methyl testosterone per lamb per day; (3) 42.5 mg. methyl testosterone per lamb per day; and (4) testosterone propionate, in oil, injected subcutaneously each 14 days at the rate of 3.3 mg. per 100 pounds of body weight per day. Both groups fed methyl testosterone gained 16 percent more than the controls, whereas the lambs injected with testosterone propionate gained 38 percent more. Feed efficiency favored the treatments with the lambs that were injected with testosterone propionate requiring 19 percent less feed per unit of gain. Carcass grades and dressing percentages were highest in the group fed the higher level of methyl testosterone and lowest in the group injected with testosterone propionate. Thyroid weights were depressed by the lower level of methyl testosterone, and increased by the injection of testosterone propionate.

Lamb Experiment 2

Method and materials. The objectives of this study were: first, to study further the effects of methyl testosterone and testosterone propionate on ewe lambs; second, to determine if testosterone propionate is effective orally in the ewe lamb; third, to study the effects of feeding a combination of methyl testosterone and stilbestrol; and fourth, to test the effect of injecting testosterone propionate into ewe lambs being fed stilbestrol.

Eight groups of ewe lambs averaging 79 pounds initially in full fleece, and of Western origin were allotted, handled and weighed as in Experiment 1. The duration of the trial was the 82-day period from March 31 to June 21, 1954.

The complete ration included, in percent: cracked corn, 39.5; ground alfalfa hay, 40; cane molasses, 15; and soybean oil meal, 5.5. During the first 14 days of the trial, while the lambs were being accustomed to feed, the mixture contained 50 percent ground alfalfa hay and 29.5 percent cracked corn with the other ingredients the same as listed above. Further adjustment was made in the ration during the last two weeks by the inclusion of more corn at the expense of hay, thus making the average ration consumed by the lambs for the entire trial, in percent: cracked corn,

39.28; ground alfalfa hay, 39.90; cane molasses, 15.48; and soybean oil meal, 5.35.

The treatments used are shown in Table 17. As in Experiment 1, the orally administered drugs were mixed with the soybean oil meal so as to provide the intake listed in Table 17 for an average feed intake of 3.50 pounds per lamb per day.

The lambs were sold to and slaughtered by the Iowa Packing Company of Des Moines, and through their cooperation warm and chilled carcass weights were obtained, government grades were determined, and the reproductive tracts were taken from the killing floor and then to Ames for study. The results of the several analyses of variance of the data are contained in the Appendix, Table 44.

Results and discussion. The weight gains and feed data are shown in Table 17. All treatment groups gained more than the control group, but the differences were small and not statistically significant. Similarly, average daily feed consumed per lamb and feed required per unit of gain favored the experimental treatments in every case, but the differences were not significant. Combinations of methyl testosterone and stilbestrol as well as the injection of testosterone propionate to lambs being fed stilbestrol had no significant effect on gains.

Table 17. Growth and fattening stimulation in ewe lambs with methyl testosterone, testosterone propionate and stilbestrol - Lamb Experiment 2[a]

Group	Additions to the basal ration	Daily gain[b]	Feed per day[b]	Feed per 100 lb. gain[b]
			(pounds)	
1	None	0.32	3.36	1050.00
2	3.3 mg. testosterone propionate per 100 lb. body weight per day[c]	0.38	3.55	934.00
3	2 mcg. stilbestrol per pound of ration	0.36	3.48	966.67
4	60 mg. testosterone propionate per lamb per day	0.36	3.47	963.89
5	Testosterone propionate as in Group 2, and stilbestrol as in Group 3	0.42	3.65	869.05
6	47 mg. methyl testosterone per lamb per day plus 2 mcg. stilbestrol per pound of feed	0.36	3.62	1005.55
7	Methyl testosterone as in Group 6 for 35 days, then stilbestrol as in Group 2 for 47 days	0.36	3.49	969.44
8	47 mg. methyl testosterone per lamb per day	0.38	3.62	952.63
	Average	0.37	3.53	963.90

[a]Averages of four lambs per group.

[b]Treatment effects not significant at P = .05 or less.

[c]Injected subcutaneously each 14th day at above rate.

The feeding of testosterone propionate at 25 times the level of the injected dose that gave a significant response in Experiment 1 produced no response in weight gain.

The data for the various carcass characteristics are shown in Table 18. Carcass grades were highly variable within groups and the differences between groups of no statistical significance, even though all experimental treatment groups contained two choice lambs, whereas the control group contained none. Dressing percent was actually lowest in the group injected with testosterone propionate, but the differences were small and indicated only that no harmful effects of treatment resulted.

Carcass shrink in chilling for a 24-hour period ranged from .9 to 1.56 percent for the different groups. These differences were of no statistical significance.

The weights of the uteri and ovaries are shown in Table 19. The various treatments had no significant effect on either the weight of the uteri or ovaries; however the effect on the uteri was just short of significance at P = .05 (2.45 against 2.49 for significance at P = .05). Both methyl testosterone and stilbestrol alone decreased the size of the uterus, but the two in combination had no effect on uterine size as compared to the control lambs. On the other hand, testosterone propionate had no effect either as an

Table 18. Cooler shrink, dressing percent, and carcass grade - Lamb Experiment 2[a]

Group	Treatment[b]	Cooler shrink[c]	Dressing percent[c]	Carcass grade[c,d]
		(percent)		
1	Control	1.22	44.40	4.75
2	Testosterone propionate injection	1.16	43.60	5.40
3	Stilbestrol	1.13	45.70	6.25
4	Testosterone propionate orally	0.63	45.50	6.00
5	Testosterone propionate plus stilbestrol	1.70	45.00	6.25
6	Methyl testosterone plus stilbestrol	0.90	45.30	6.50
7	Methyl testosterone for 35 days, then stilbestrol for 47 days	1.56	45.50	6.75
8	Methyl testosterone	1.35	45.50	6.25
	Average	1.19	45.06	6.02

[a,b]Explained in Table 17.

[c]Treatment effects not significant at P = .05 or less.

[d]Carcass grades computed using the following numerical system: choice, 8; choice minus, 7; good plus, 6; good, 5; good minus, 4; utility plus, 3; and utility, 2.

Table 19. The effect of methyl testosterone, testosterone propionate, and stilbestrol on the weights of ovaries and uteri - Lamb Experiment 2[a]

Group	Treatment[b]	Uteri[c]	Ovaries[c]
		(grams)	
1	Control	22.26	0.8903
2	Testosterone propionate injected	23.54	0.9357
3	Stilbestrol	18.21	0.7427
4	Testosterone propionate orally	23.23	1.0700
5	Testosterone propionate plus stilbestrol	14.62	0.7025
6	Methyl testosterone plus stilbestrol	22.33	0.8996
7	Methyl testosterone for 35 days, then stilbestrol for 47 days	22.45	0.8502
8	Methyl testosterone	13.58	1.0414
	Average	20.02	1.0414

[a,b]Explained in Table 17.

[c]Treatment effect not significant at P = .05 or less.

injection or when fed, but when injected into lambs being fed stilbestrol the size of the uterus was reduced even more than from stilbestrol alone. These effects are difficult to correlate in the light of present knowledge, but do suggest that the effect on the uterus is difficult to

predict in the ewe lamb.

Summary. Thirty-two ewe lambs of Western origin, in full fleece, and averaging 79 pounds in weight were allotted from outcome groups by weight to eight groups for an 82-day trial conducted in the March to June period of 1954. The lambs were individually fed an 11.5 percent protein basal ration of cracked corn, soybean oil meal, cane molasses, and alfalfa hay. Additions to the basal ration consisted of: (1) none; (2) testosterone propionate, in oil, injected each 14 days at the rate of 3.3 mg. per 100 pounds of body weight; (3) 2 mcg. stilbestrol per pound of feed; (4) 60 mg. testosterone propionate per lamb daily; (5) testosterone propionate injected as in Group 2, to lambs being fed stilbestrol as in Group 3; (6) 47 mg. methyl testosterone per lamb per day plus 2 mcg. stilbestrol per pound of feed; (7) 47 mg. methyl testosterone per lamb per day for 35 days, then 2 mcg. stilbestrol per pound of feed for 47 days; (8) 47 mg. methyl testosterone per lamb per day.

All treatment groups gained more than the control lambs, but the differences were small and of no statistical significance. The feeding of testosterone propionate at 25 times the injected dose that had produced a significant response in Experiment 1 was without effect. Dressing percent, carcass shrink, and carcass grades showed only small

variation and the differences between treatments were not significant. Uterine weights were reduced by either stilbestrol or methyl testosterone alone, but a combination of the two had no effect on the size of the uterus.

Lamb Experiment 3

Method and materials. This trial represents a continuation of the study of the effects of methyl testosterone, testosterone propionate, and stilbestrol on ewe lambs being finished for market.

Six groups of six ewe lambs each were allotted from outcome groups by weight and assigned to six experimental treatments in a randomized incomplete block design. The lambs were of Western origin, in full fleece, excellent in quality and appeared to be by Hampshire rams. The initial weight of all groups was 74 pounds. The handling, allotment and weighing of the lambs was the same as in Experiment 1. This trial was conducted from October 11, 1954 to January 19, 1955, a period of 84 days.

The basal ration was the same as was used in Experiment 2. The stilbestrol and methyl testosterone were mixed with the ration to provide a definite amount of each per pound of total ration, but the amounts actually consumed are expressed as amounts per lamb per day so that direct

comparison with the other trials in the series can be made. The treatments used in this experiment are shown in Table 20.

The slaughtering, collection of carcass data, and the recovery of the reproductive tracts, were handled as in Experiment 2.

Table 20. Growth and fattening stimulation in ewe lambs with testosterone propionate, methyl testosterone, and stilbestrol - Lamb Experiment 3[a]

Group	Additions to the basal ration	Daily gain[b]	Feed per day[b]	Feed per 100 lb. gain[b]
			(pounds)	
1	None	0.44	3.70	840.91
2	3.3 mg. testosterone propionate per 100 lb. body weight per day[c]	0.35	3.31	945.71
3	39.24 mg. methyl testosterone per lamb per day	0.39	3.27	838.46
4	2.06 mg. stilbestrol per lamb per day	0.40	3.55	887.50
5	Testosterone propionate as in Group 2, plus stilbestrol as in Group 4	0.40	3.35	837.50
6	Methyl testosterone as in Group 3 plus stilbestrol as in Group 4	0.43	3.61	839.53
	Average	0.40	3.47	867.50

[a]Average of six lambs per group.

[b]Treatment effects not significant at P = .05 or less.

[c]Injected subcutaneously each 14th day at above rate.

Results and discussion. The group averages for daily gains, daily feed consumed, and feed efficiency are given in Table 20. Neither the feeding of methyl testosterone or stilbestrol, alone or in combination, nor the injection of testosterone propionate, alone or to lambs being fed stilbestrol, produced a significant effect on rate of gain as compared to the control lambs. The lambs receiving no drug additive gained slightly more than any treatment group, but the differences show no statistical significance. Similarly, feed consumption was reduced slightly by treatment and feed efficiency was almost identical in all groups except for the group that was injected with testosterone propionate. This group gained the least and required 104.8 pounds more feed per 100 pounds of gain than the control lambs. All other groups, including the control lambs, were in the range of 837.5 to 840.9 pounds of feed required per 100 pounds gain.

The data for dressing percent, cooler shrink, and carcass grades are shown in Table 21. The averages for the different groups show only small variation, and those for cooler shrink show no statistical significance. However, the carcass grades indicate again the slight lowering of grade by the injection of testosterone propionate, as these lambs and those fed a combination of methyl testosterone

Table 21. Dressing percent, cooler shrink, and carcass grade - Lamb Experiment 3[a]

Group	Treatment[b]	Dressing percent[c]	Cooler shrink[c]	Carcass grade[c,d]
		(percent)		
1	Control	50.6	2.40	9.2
2	Testosterone propionate injection	49.4	1.98	8.0
3	Methyl testosterone	50.1	2.26	8.8
4	Stilbestrol	50.0	2.68	8.8
5	Testosterone propionate and stilbestrol	50.0	2.31	8.7
6	Methyl testosterone and stilbestrol	48.7	2.75	8.5
	Average	49.8	2.40	8.67

[a,b]Explained in Table 20.

[c]Treatment effect not significant at P = .05 or less.

[d]Carcass grades computed using the following numerical system: prime, 11; prime minus, 10; choice plus, 9; choice, 8; choice minus, 7; good plus, 6; good, 5; and good minus, 4.

and stilbestrol were the only groups not yielding one or more carcasses of prime grade. All lambs in the experiment graded either choice or prime, but one and this exception was in the group injected with testosterone propionate. In Experiment 1 the group of lambs that had been injected with testosterone propionate showed the greatest cooler

shrink, and a similarly treated group in this experiment showed the least.

The average weight of the uteri, ovaries, and the average number of follicles per group are shown in Table 22. Testosterone propionate injected at the rate of 3.3 mg. per 100 pounds of body weight per day significantly increased

Table 22. The effects of methyl testosterone, testosterone propionate, and stilbestrol on the weight of the uteri, and weight and follicle development of the ovaries - Lamb Experiment 3[a]

Group	Treatment[b]	Uteri[c]	Ovaries	Follicles[d]
		(grams)		(number)
1	Control	24.04	1.22	1.83
2	Testosterone propionate injected	29.18	1.16	1.16
3	Methyl testosterone	18.57	1.07	1.20
4	Stilbestrol	24.34	1.04	0.00
5	Testosterone propionate and stilbestrol	22.01	0.99	0.00
6	Methyl testosterone and stilbestrol	15.78	0.68	0.00
	Average	22.32	1.03	0.70

[a,b]Explained in Table 20.

[c]Treatment effect significant at P = .01.

[d]Only follicles over 2 mm. in diameter counted, but lambs in Group 6 had no observable follicles of any size.

uterine size as compared to the ewe lambs in the control group, whereas either methyl testosterone fed alone or in combination with stilbestrol significantly reduced the average size of the uterus. This trend was noted in Experiment 2, but in that case the differences were not statistically significant. In this experiment stilbestrol had no effect on the size of the uterus, yet tended to reduce the size of the ovary and its use resulted in a complete absence of follicles of 2 mm. size or above. The effect of stilbestrol on the reduction in the number of follicles seems to be a property of stilbestrol as the effects of either androgen plus stilbestrol seem additive in reducing both ovary size and follicle count. On the other hand, the reduction in the size of the uterus seems to be an action of methyl testosterone and this action was not checked by combining methyl testosterone with stilbestrol in this experiment. In Experiment 2 either methyl testosterone or stilbestrol reduced uterine size alone, but not in combination with each other. In Experiment 2, however, the level of stilbestrol feeding was only 2 mcg. per pound of feed as compared to 600 mcg. per pound of feed in the present trial.

An estimate of the development of the mammary gland was made by observation of this gland in the chilled carcass and arbitrarily assigning a numerical value representing

the degree of enlargement. According to this measure, the mammary glands were smallest in the control group, with each treatment effect being that of stimulating mammary size by 25 percent.

Summary. Thirty-six ewe lambs of Western origin, in full fleece, and averaging 74 pounds in weight were randomly allotted to six groups for an 84-day trial conducted in the October to January period of 1954-1955. The lambs were individually fed an 11.5 percent protein basal ration of cracked corn, soybean oil meal, cane molasses, and ground alfalfa hay. Additions to the basal ration consisted of: (1) none; (2) testosterone propionate, in oil, injected at the rate of 3.3 mg. per 100 pounds live weight per day; (3) 12 mg. of methyl testosterone per pound of feed; (4) testosterone propionate injection (as in Group 2) and 600 mcg. of stilbestrol per pound of feed; and (6) 12 mg. of methyl testosterone plus 600 mcg. of stilbestrol per pound of feed.

Differences in average daily gains, feed consumed per day, and feed efficiency were small and of no statistical significance. All lambs in the experiment graded either choice or prime, but one, and this exception was from the group that had been implanted with testosterone propionate. This group, and that representing the combination of methyl testosterone and stilbestrol, were the only groups not

yielding one or more prime carcasses.

Testosterone propionate injected at the rate of 3.3 mg. per 100 pounds of body weight per day significantly increased uterine size as compared to the control lambs, whereas methyl testosterone fed alone or in combination with stilbestrol significantly reduced the size of the uterus. Stilbestrol, fed alone or in combination with either androgen, reduced follicle size to the extent that none of 2 mm. size were present in these groups as compared to an average of 1.8 follicles of this size or larger in the control group.

Lamb Experiment 4

Method and materials. One hundred ewe lambs of Western origin averaging 71.7 pounds in weight were used for a 71-day group feeding trial conducted in the February to May period of 1955. They were assigned from outcome groups by weight to ten groups of ten lambs each, then two groups of ten combined to form the five experimental groups of 20 lambs each. One ten-head sub-group was then randomly selected and shorn two days before the start of the trial. The remaining ten lambs in each group were left in fleece that was estimated to be a number two pelt. The lambs were vaccinated for enterotoxemia, ear-tagged, paint stamped,

and weighed as in previous experiments in this investigation. Iodized salt, in block form, was placed in all lots and water was provided by hand-watering twice daily.

The lambs were fed twice daily on cracked corn, ad libitum; 44 percent soybean oil meal, .25 pound; and alfalfa hay. After 28 days of feeding the alfalfa hay was limited to 1.5 pounds per lamb per day and refused hay weighed back before the next feeding. Additions to the basal ration consisted of: (1) none; (2) 2.5 mg. stilbestrol per lamb per day; (3) 3.5 mg. stilbestrol per lamb per day; (4) a commercial implant (Synovex) of 250 mg. progesterone and 10 mg. estradiol; and (5) a 30 mg. testosterone propionate implant.

The two groups receiving the stilbestrol were fed this compound thoroughly mixed with the soybean oil meal. The soybean oil meal, or soybean oil meal-stilbestrol premix, was fed first to all lots, then the corn was fed in the same sequence of lots, and the hay was fed after all or most all of the corn had been consumed.

A yearling ram was purchased and vasectomized for the purpose of determining if any treatment effect would result in ewe lambs accepting the ram.

The lambs were slaughtered through the Iowa Packing Company as were those in Experiments 2 and 3. Warm and

chilled carcass weights were obtained and the reproductive tracts were taken from the killing floor as in previous experiments.

The several groups of data for which individual values were available were analyzed statistically and the results are shown in the Appendix, Table 46.

Results and discussion. The data for average daily gain, feed intake, and feed efficiency are shown in Table 23 and graphically in Figure 32. Ewe lambs implanted with a progesterone-estradiol combination made 14.6 percent more gain than the control lambs, and produced 100 pounds of gain on 588.8 pounds of feed - 110 pounds less than the control lambs. The lambs that were implanted with the progesterone-estradiol combination consumed an average of 1.56 pounds of corn per day as compared to 1.51 pounds for the control lambs; however the treated lambs refused more hay and the total feed consumed for each group was the same - 3.25 pounds per lamb per day.

Ewe lambs fed either 1.54 or 2.67 mg. of stilbestrol per head per day gained 12.5 percent less than the control lambs, were more difficult to keep on feed, and consumed less feed per day. At no time after the lambs were on a full feed of corn would either group receiving stilbestrol consume as much corn as the control lambs, and the lambs fed

Table 23. Growth and fattening stimulation with stilbestrol, a progesterone-estradiol implant, and a testosterone propionate implant - Lamb Experiment 4

Group	Additions to the basal ration	Lambs	Daily gain[a]	Feed per day				Feed per 100 lb. gain
				Corn	Soybean oil meal	Alfalfa hay	Total	
		(number)		(pounds)				
1	None	19[b]	0.48	1.51	0.25	1.49	3.25	699.11
2	1.4 mg. stilbestrol per lamb per day	20	0.42	1.41	0.25	1.44	3.10	734.44
3	2.67 mg. stilbestrol per lamb per day	18[c]	0.42	1.36	0.25	1.39	3.00	712.29
4	Progesterone-estradiol implant[d]	20	0.55	1.56	0.25	1.44	3.25	588.92
5	30 mg. testosterone propionate	20	0.46	1.48	0.25	1.45	3.18	695.39
	Average		0.47	1.46	0.25	1.44	3.16	686.01
	L.S.D. at .05		0.054					

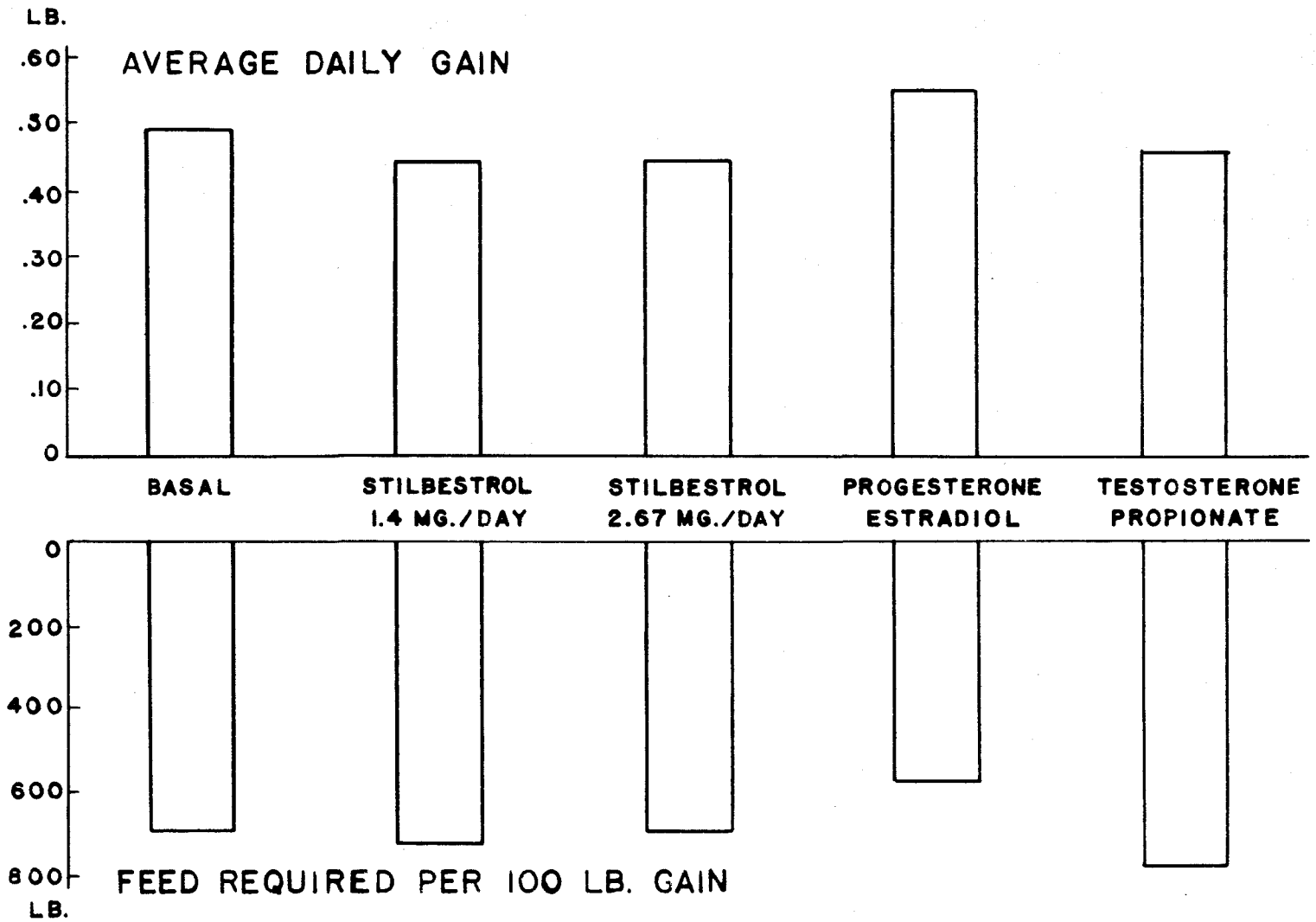
[a]Treatment effect significant at P = .05 or less.

[b]One lamb removed as she lambed during the trial.

[c]One lamb removed as she lambed during the trial, and another lamb died of unknown cause.

[d]Advertised to contain 250 mg. progesterone and 10 mg. estradiol.

Figure 32. The effect of treatment on average daily gain and feed required per 100 pounds of gain - Lamb Experiment 4


LB.
AVERAGE DAILY GAIN
.60
.50
.40
.30
.20
.10
0
BASAL
STILBESTROL 1.4 MG./DAY
STILBESTROL 2.67 MG./DAY
PROGESTERONE ESTRADIOL
TESTOSTERONE PROPIONATE
0
200
400
600
800
LB.
FEED REQUIRED PER 100 LB. GAIN

stilbestrol consistently refused more of the daily allowance of 1.5 pounds of alfalfa hay per lamb.

The two groups that were fed stilbestrol were started on levels of 2 and 3.5 mg. of stilbestrol per lamb per day for Groups 2 and 3, respectively, but because of difficulty in keeping the lambs on feed the levels were dropped on the 28th day of the trial to 1 and 1.75 mg., respectively. Group 2 remained at the 1 mg. per head per day level throughout the remainder of the trial, whereas Group 3 was increased to 2.5 mg. on the 49th day of the trial. Feed consumption improved with the initial reduction in level of stilbestrol feeding, and did not seem to be affected by the increase made for Group 3 as of the 49th day.

Implanting ewe lambs with a 30 mg. pellet of testosterone propionate had no significant effect on rate of gain, and the lambs so treated were almost identical to the control lambs in feed consumption and feed efficiency. This experiment is the third of four in this investigation in which testosterone propionate has been shown to have no effect on rate of gain in ewe lambs. In Experiment 1, a significant improvement in rate of gain resulted from the implantation of testosterone propionate at the rate of 3.3 mg. per 100 pounds body weight. These results were not duplicated in either Experiment 2 or 3. It was observed

that the lambs implanted with testosterone propionate in this experiment were easier to keep on feed than the control lambs.

The shearing of ewe lambs that had previously been shorn some 90 days prior to the start of the experiment, and which were estimated to have a number two pelt, resulted in a small but not significant improvement in rate of gain. The average gains by groups are shown in Table 24. The lambs that were shorn yielded an average wool clip of three pounds additional weight in the pre-experimental period of allotment, shearing, and weighing. Adjusting for this additional shrink practically equalizes all within group gains. The over all average daily gain for wooled lambs of .45 pound is then compared to a .46 pound average for shorn lambs rather than the gain plus fill average of .49 pound for the shorn lambs. In these comparisons the shorn lambs that were implanted with testosterone propionate gained .52 pound daily compared to .40 pound daily for their wooled pen mates, and this value for the shorn lambs is .51 pound per day after making the correction for extra shrink. This difference in favor of the shorn lambs for this treatment is not easily explained, but is somewhat suggestive of an increased metabolic rate due to treatment; if this were the case, shearing should

Table 24. Actual gains in the experimental period compared with gains corrected for the extra shrink of shearing one-half of each group, and a comparison of dressing percentages - Lamb Experiment 4[a]

Group	Treatment[b]	Daily gain			Dressing percent		
		Wooled	Shorn	Shorn[c]	Wooled	Shorn	Average
		(pound)					
1	Control	0.46	0.51	0.48	47.25	48.06	47.64
2	Stilbestrol	0.42	0.43	0.40	46.76	48.61	47.74
3	Stilbestrol	0.41	0.43	0.39	46.88	49.92	48.35
4	Progesterone-estradiol implant	0.55	0.56	0.54	46.35	48.61	47.48
5	Testosterone propionate implant	0.40	0.52	0.51	47.07	48.44	47.77
	Average	0.45	0.49	0.46	46.86	48.73	47.80

[a,b]Explained in Table 23.

[c]Corrected for the extra shrink in shearing above the wool clip.

have aided in the elimination of heat from the body. However, the shorn lambs implanted with testosterone propionate, even though gaining .11 pound more daily than their wooled pen mates were not significantly different in this measure of response than shorn lambs in the control group which gained an average of .48 pound daily. Rectal temperatures of a number of lambs, as of May 2 with an environmental temperature of 88 degrees Fahrenheit, revealed no trend favoring lower body temperature in the control lambs. However, in all groups except the controls, the shorn lambs had the lowest body temperature - the difference ranged from one-half to six-tenths of a degree.

The data for carcass characteristics are contained in Tables 24 and 25. The implantation of ewe lambs with a progesterone-estradiol implant significantly decreased carcass grades and 50 percent of the carcasses were classed as yearlings as compared to 100 percent lamb carcasses in the control group. The lambs were no doubt approaching a year of age when slaughtered; hence nearing the time when one would expect a percentage of yearling carcasses to appear normally. It would seem that the progesterone-estradiol implant hastened maturity and this effect upon ewe lambs approaching a year of age was quite apparent in carcass appearance and classification. This finding agrees

Table 25. Cooler shrink, carcass grade, and other carcass characteristics - Lamb Experiment 4[a]

Group	Treatment[b]	Cooler shrink			Carcass grade[c,d]	Grading as yearlings	Grading as soft
		Wooled	Shorn	Average			
		(percent)				(number)	(number)
1	Control	2.92	2.86	2.89	5.89	0	2
2	Stilbestrol	3.03	2.49	2.76	5.65	6	0
3	Stilbestrol	3.00	2.68	2.84	5.94	6	4
4	Progesterone-estradiol implant	3.06	2.98	3.02	4.80	10	5
5	Testosterone propionate implant	2.55	2.44	2.49	6.00	1	0
	Average	2.91	2.69	2.80	5.66		
	L.S.D. at .05			0.45	0.35		

[a,b]Explained in Table 23.

[c]Significant at P = .05 or less.

[d]Carcass grades computed using the following numerical system: choice, 8; choice minus, 7; good plus, 6; good, 5; good minus, 4; utility plus, 3; and utility, 2.

with the work of Acker *et al*. (1) as does the observation that the pelts were very difficult to remove from lambs so treated. These workers explain this as being due to extra connective tissue immediately beneath the skin. Twenty-five percent of the control lambs produced carcasses grading choice with none lower than low good, whereas the group implanted with progesterone and estradiol produced no choice, and 15 percent utility grade, carcasses when all were graded on the lamb basis.

Bell *et al*. (10) found that implantation with progesterone and stilbestrol was more detrimental to carcass grade than was stilbestrol alone. From Table 25 it can be observed that ewe lambs fed stilbestrol produced 30 and 33.3 percent yearling carcasses for the 1.4 and 2.67 mg. per lamb per day levels, respectively, but this is the first of three experiments in which this feature of stilbestrol was observed with ewe lambs. Ewe lambs fed stilbestrol at either level used in this experiment, or at any level used in other experiments in this series, have been difficult to pelt; however the degree of difficulty as judged by the number of torn carcasses coming from the killing floor is less than that for ewe lambs implanted with progesterone and estradiol as used in this experiment.

A 30 mg. pellet of testosterone propionate for ewe

lambs fed for a 71-day period was without effect on carcass grade. This agrees with the findings of Experiments 2 and 3 in which carcass grades were not affected by testosterone propionate in trials in which its injection had no effect on rate of gain.

The fourth day after implantation with progesterone and estradiol excessive riding was evident in the ewe lambs. This condition persisted in day to day varying degrees of intensity for the first half of the trial, but was observed only occasionally thereafter. The first evidence of riding occurred on March 4 and on March 9 the vasectomized ram served the first lamb. This lamb accepted the ram again on both March 9 and 10. Another lamb was served on March 14, 16, and 21, and on April 23. In all, 20 percent of the lambs accepted the ram one or more times in the March 9 to April 23 period. The lambs in the control group as well as those in the two groups being fed stilbestrol would not accept the ram. One lamb in the testosterone propionate group did accept the ram, but no evidence of riding was observed in this lot.

By the 50th day of the trial it was obvious that the lambs implanted with progesterone and estradiol were developing an appearance of growthiness and over all lack of the smoothness exhibited by the other groups. Prominence of the

hip bones and sunken loins were the most obvious characteristics contributing to an aged appearance.

The data on the condition and weight of the uteri and ovaries are shown in Table 26 and these same data are presented graphically in Figure 33. Stilbestrol tended to increase uterine size and decrease ovary size progessively with the level of feeding. The progesterone-estradiol implant increased uterine size by 29 percent without affecting the size of the ovaries. The implant of testosterone propionate was without effect on uterine weights, but increased both the size of the ovary and the size and number of follicles. The feeding of an average of 1.4 mg. of stilbestrol per lamb daily decreased ovary size and reduced the number of follicles of 2 mm. diameter or larger by 92 percent, whereas the higher level of 2.76 mg. of stilbestrol per lamb daily reduced ovary size by 33.5 percent and follicles of 2 mm. or larger to zero. Treatment with progesterone and estradiol retarded follicle development and at the time of slaughter no follicles of 2 mm. or larger were present.

Summary. One hundred ewe lambs of Western origin and averaging 71.7 pounds in weight were allotted from outcome groups by weight to five groups of 20 head each for a 71-day trial conducted in the February to May period of 1955. Ten

Table 26. Weight and condition of the uteri and ovaries - Lamb Experiment 4[a]

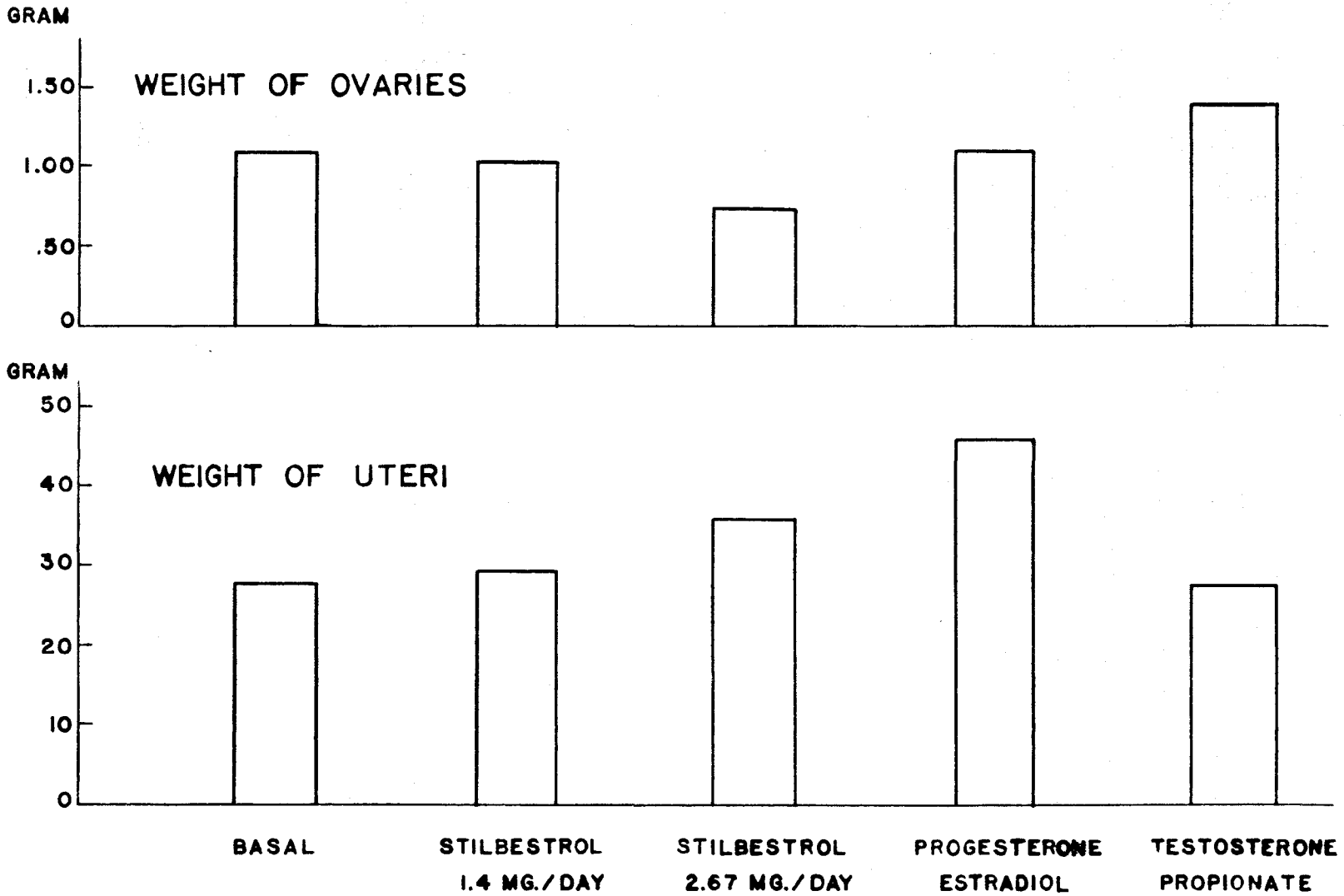
Group	Treatment[b]	Uteri[c]			Ovaries[b]	
		Weight	Normal	Inflamed	Weight	Follicles[d]
		(grams)	(number)		(grams)	(number)
1	Control	27.74	19	0	1.11	1.22
2	Stilbestrol	29.58	15	4	1.03	0.15
3	Stilbestrol	35.65	10	10	0.70	0.00
4	Progesterone-estradiol implant	45.47	11	9	1.10	0.00
5	Testosterone propionate implant	27.67	20	0	1.42	1.84
	Average	33.24	15	4.6	1.07	0.64
	L.S.D. at .05	4.45			0.28	

[a,b]Explained in Table 23.

[c]Treatment effects significant at P = .05 or less.

[d]Only follicles of 2 mm. or more in diameter counted.

Figure 33. Effect of treatment on the weight of ovaries and uteri - Lamb Experiment 4


GRAM
WEIGHT OF OVARIES
1.50
1.00
.50
0
GRAM
WEIGHT OF UTERI
50
40
30
20
10
0
BASAL
STILBESTROL 1.4 MG./DAY
STILBESTROL 2.67 MG./DAY
PROGESTERONE ESTRADIOL
TESTOSTERONE PROPIONATE

lambs within each group were randomly selected and shorn two days before the start of the trial. The remaining 10 lambs in each group were left in fleece which was estimated to be a number two pelt. The lambs were fed twice daily on cracked corn, ad libitum; 44 percent soybean oil meal, .25 pound; and alfalfa hay, 1.5 pounds. Additions to the basal ration consisted of: (1) none; (2) 1.4 mg. stilbestrol per lamb per day; (3) 2.67 mg. stilbestrol per lamb per day; (4) a commercial implant (Synovex) of progesterone and estradiol; and (5) a 30 mg. implant of testosterone propionate.

Ewe lambs implanted with a progesterone-estradiol combination gained 14.6 percent more and produced a unit of gain on 16 percent less feed than control lambs receiving no sex hormone compound. This treatment reduced carcass grades significantly and produced 50 percent yearling class carcasses. A level of either 1.4 or 2.67 mg. of stilbestrol per lamb per day decreased food intake, rate of gain, and produced 30 and 33.3 percent yearling carcasses, respectively.

A 30 mg. implant of testosterone propionate had no effect on food intake, rate of gain, or average carcass grade.

Excessive riding was evident in the group implanted with progesterone and estradiol by the fourth day of the

trial and 20 percent of the lambs in this group accepted a vasectomized ram at least once. Some lambs in the group were served on as many as four different dates during the first half of the trial.

The progesterone-estradiol implant enlarged uterine size significantly and reduced the size and number of follicles, but had no effect on average weight of the ovaries. Stilbestrol, on the other hand, increased uterine size, decreased ovary size and was detrimental to follicle size and development. Testosterone propionate had no effect on uterine size, but increased both the weight of the ovary and the number of follicles measuring 2 mm. or more in diameter.

Shorn lambs gained faster than their wooled pen mates in every group, but correcting these values for the extra shrink which accompanied shearing erased the advantage for shearing in all but the group implanted with testosterone propionate. In this case, the shorn lambs gained 27 percent faster than their wooled pen mates, but failed to gain significantly more than the shorn control lambs.

GENERAL DISCUSSION

The primary interest of the animal nutritionist in the synthetic sex hormones is in their effects that produce more rapid gains and more economical use of feed in the production of meat, but these effects are not easily obtained in all species without also stimulating the reproductive organs, mammary glands and teats, as well as other organs of the body. Thus far, the beef steer is in a class by itself in giving quite phenomenal and consistent response in rate of gain and in feed efficiency at a level of stilbestrol feeding that produces negligible effects of an undesirable nature, Iowa (22, 23, 24, and 25), Purdue (8, 64), Nebraska (5), Colorado (32), Ohio (52), and Tennessee (9). Beef heifers are stimulated, but to a lesser extent, Burroughs et al. (23) and Clegg and Cole (29).

In response to stilbestrol, the pig and the lamb are apparently different from the beef animal. In these investigations the feeding of stilbestrol to growing-finishing pigs at levels of from 5 to 1280 mcg. per pound of feed, in two experiments, produced no significant increase in rate of gain or feed efficiency. The feeding of stilbestrol did produce a significant increase in size of the reproductive tract, in the diameter of the cervix,

and a reduction in the size of the ovaries. Observations on the development of teats and enlargement of the vulva also indicate estrogenic stimulation.

The observations on the transverse diameter of the pelvic inlet and the diameter of the cervix have not been reported previously for the pig, but the other observations and findings agree with those of Perry et al. (63), Beeson et al. (7), and Braude (15). Levels of .003 to 50 mg. of stilbestrol have been fed per pig per day, in the United States and in Great Britain, without significant improvement in rate of gain, even though trends toward this end have been observed. In the matter of response to stilbestrol the pig seems to be intermediate between the rat with which food intake and growth are depressed, Preston (67), and the beef steer with which feed intake and rate of growth are increased, Burroughs et al. (22). Thus far no one has explained or demonstrated the difference in the two species in this regard and this investigation did not yield the answer. Since the pig, like the rat, is a monogastric animal the work of Meites (56), showing that the administration of large doses of estrogens aggravated pre-existent deficiencies of vitamin B_{12} or thiamine, may have a connection to the lack of response of the pig to stilbestrol administration. These symptoms could be alleviated, in the

rat, by supplementation of the diet with 5 to 10 times the normal requirement of these vitamins. It is well established that the thiamine requirement is materially increased during pregnancy, Peterson and Strong (65), and since some of the responses of the pig could be interpreted as suggesting that the hormone level of pregnancy was approached by the feeding of stilbestrol, it would seem logical that a study of thiamine or thiamine and other B vitamins might be considered in future work. This is all based on the speculation that one of the reasons the pig does not respond to stilbestrol feeding, or implantation, by increased rate of growth is that some dietary factor may be limiting the response.

The fact that lipothiamide pyrophosphate, a complex of thiamine and lipoic acid, is a necessary catalyst in the oxidative decarboxylation of pyruvic acid and alpha ketoglutaric acid to form acetyl coenzyme A or succinyl coenzyme A by enzyme preparations from Escherichia coli, Fruton and Simmonds (37), is another possibility. It could possibly be concerned in the action of stilbestrol in the pig. Then, too, as Meites (57) suggests, vitamin B_{12} may be involved. It may also be that stilbestrol has a different effect on the pituitary of the pig than it has in the beef animal.

In these trials stilbestrol did not stimulate weight gain in the ewe lamb at levels of .003 to 3.5 mg. per head per day. This agrees, in part, with Hale et al. (42) who failed to duplicate, subsequently, the stimulation received in two earlier trials, but the lambs used were largely wethers. Acker et al. (1) report a 15 percent improvement in gain and in feed efficiency from the feeding of 2 mg. of stilbestrol per head daily to mixed ewe and wether lambs, but the proportion of the sexes was not given.

This investigation shows that levels of either 1.4 or 2.67 mg. of stilbestrol per head daily significantly depressed rate of gain as compared to control ewe lambs. This reduction can be accounted for largely by the reduction in feed intake as efficiency of feed conversion was not altered significantly. One group of 20 ewe lambs fed 3.5 mg. of stilbestrol per head daily showed evidence of digestive disorders, nausea, and were difficult to keep on feed. Reduction of the level of stilbestrol to 2.5 mg. improved feed intake as compared to the higher level and the lambs were then easier to keep on feed, but their feed consumption never equalled that of the control lambs. Shorr et al. (72) observed, in 1939, that stilbestrol was potent orally, but that its oral administration caused nausea and intestinal distress in 25 percent or more of

human patients, and that generally the symptoms of distress were alleviated when the dose was reduced. They failed to show, as others had suggested, that the distress was lessened by injection rather than the oral use of stilbestrol. Work by Story (77) indicates that lambs implanted with stilbestrol consumed but little feed for the first 14 days after implantation, but then recovered their appetite and consumed more total feed than the control lambs for the entire trial.

Recently Clegg et al. (31) reported that the stimulation in rate of gain in lambs from the implantation of stilbestrol was independent of age, sex, or dietary regime. These investigators used a 12 mg. pellet of stilbestrol regardless of the age, sex, or weight of the lamb. Thus one is led to wonder if a part of the lack of response in rate of gain in the feeding of stilbestrol to ewe lambs is not due to the fact that stilbestrol, by oral administration, indirectly produces nausea and intestinal distress and hence reduces feed consumption in a variable percentage of animals in most groups of feeder lambs. Brooks et al. (19) found that sheep fed 20 mg. of stilbestrol per head daily developed anorexia during the first week of feeding. If this speculation is correct it might be concerned with the variable results obtained by different workers in the same season of the year.

Again thiamine or thiamine and other B vitamins might be worth investigating. It is more difficult, of course, to understand how a ruminant animal such as the sheep could show a response to thiamine or other B vitamin supplementation, but we understand so little as to the mode of action of stilbestrol or the stress it produces that there are many possibilities. Bentley et al. (12) report improved rate of gain in beef cattle with a supplement composed of valeric acid, biotin and para-aminobenzoic acid, whereas one of valeric acid without the B vitamins was without effect in stimulating gains.

That stilbestrol exerts some of its action through the stimulation of the anterior pituitary seems well accepted, Turner (81), Burrows (26), Clegg and Cole (29), and Fry et al. (38); however, Hormones (48) points out that the effects of estrogens on the pituitary fall into two classes, those produced by short and those produced by chronic treatment. In chronic treatment the stores of gonadtrophins are greatly reduced, whereas in short time treatments the effects are those of gonadal enlargement. Our investigations show both effects. The 10 mcg. of stilbestrol per pound of ration level with swine increased, whereas all other levels decreased, the size of the ovary. In the sheep trials the effects ranged from none to that of a

depression in ovary size. Clegg and Cole (29) suggested from work with cattle that stilbestrol administered as an implant may stimulate the pituitary which in turn may stimulate the adrenal cortex and the output of androgens may thus be increased. They observed greater nitrogen retention and the development of a masculine appearance and behavior in both steers and heifers. We observed no symptoms, in lambs or pigs, that could be classed as typically masculine, and Acker et al. (1) found no greater digestion of protein from the feeding of 2 mg. of stilbestrol per head daily to lambs; however an increase in the digestibility of protein did not accompany the retention of nitrogen prompted by stilbestrol implantation according to Clegg and Cole (29) and Galloway et al. (39). Kochakain (53) has tentatively interpreted the changes in size and enzyme activity of the kidney without similar changes in the liver or intestines as an indication that the anabolic properties of the androgens are mediated, at least in part, through the kidney. This indication, if verified, would tend to support the androgen hypothesis of Clegg and Cole (29). Fry et al. (38) concluded from work with rats that the effect of stilbestrol in increasing carbohydrate levels and nitrogen excretion is brought about by increased secretory activity of the anterior pituitary which augments hormonal production of the adrenal cortex. That

different adrenal cortex hormones may be stimulated in different species would seem to be a possibility. That species may differ in the proportion of major adrenal cortex hormones is a possibility for study. Whether the oral administration of stilbestrol stimulates nitrogen retention without increasing protein digestibility as does the implantation of the compound has not appeared in the literature to date and is a point which should be determined.

The results with the oral use of stilbestrol with lambs in the important matter of economy of gain are so variable and the optimum level of feeding so poorly defined that the long anestrus period of the ewe must be considered in thinking of all possible causes of the variable results. For example Acker et al. (1) fed lambs of both sexes for 92 days, starting November 5, 1954, and produced a 15 percent improvement in rate of gain from approximately the same level of stilbestrol as that which produced a significant depression in rate of gain in our work. However, our trial started on February 28, 1955. Thus Acker et al. (1) included in their trial the period in which a percentage of ewe lambs would have a seasonal estrus period, whereas our trial was not started sufficiently early to include this period. That such a period existed for the lambs used is shown by the fact that two head lambed from mid-November breeding

and two others were some four months pregnant when slaughtered on May 11. Kammlade et al. (51) found that the anestrus period was characteristically one of an imbalance between the follicle stimulating hormone (F.S.H.) and the lutenizing hormone (L.H.) - the F.S.H. being higher and the L.H. lower than normal. Estrogens usually inhibit the secretion of F.S.H., according to Bogart et al. (13), and our trials showed few follicles at the higher levels of stilbestrol feeding. Thus the administration of stilbestrol, in this period, should work in the direction of balance but it may be that the levels of both F.S.H. and L.H. are then too low, and it could be that ovary of the ewe in the anestrus period is incapable of producing progesterone to assist in establishing an estrogen-progesterone ratio favorable to increased gains. The lutenizing hormone is known to be involved in the secretion of progesterone, Brody (18). If this line of reasoning is developed a step further, then it may be that a level of stilbestrol which so reduces the ovary in size that its activity is diminished may be incapable of producing a stimulation in rate of gain. A degenerated ovary that is devoid of corpora lutea could be a poor source of progesterone production. Nelson and Evans (60), working with rats, concluded that injections of estrone alone stimulates the ovarian production of

progesterone. In our investigations, the feeding of 2 mg. of stilbestrol or more per head daily reduced ovary size by 15 and 63 percent in two experiments, whereas Bell *et al.* (10) found an actual increase in ovary width and length from implanting 6, 12, or 15 mg. of stilbestrol and report some stimulation in rate of gain due to treatment.

Some support of the speculation that progesterone is involved is found in that thus far no one has failed to stimulate rate of gain in lambs by progesterone-estradiol or progesterone-stilbestrol implants, O'Mary *et al.* (61, 62), Galloway *et al.* (39), Bell *et al.* (10), Bush *et al.* (27), and workers at the Michigan station (58, 59). Extending this speculation to wether lambs would necessitate the inclusion of the adrenal cortex as the source of progesterone.

The fact that no estrogenic activity was detectable in the lean or fat of pigs fed as much as 1280 mcg. of stilbestrol per pound of feed up to the time of slaughter indicates that the compound is not stored in these tissues of the pig. Slaughtering the pigs without withholding the feed containing stilbestrol should duplicate, in a sense, the slaughtering of animals implanted with stilbestrol pellets providing the pellet had not been completely absorbed prior to slaughter. Thus, our findings for the pig do not agree

with those of Stob et al. (75) for beef muscle or lamb tissue - both from animals that had been implanted. Whereas they report some, we found no estrogenic activity. Our results agree with those of Beeson et al. (7), and Braude (17). On the other hand, the finding that the liver from pigs fed 10 mcg. of stilbestrol per pound of feed had no estrogenic activity regardless of the time the pig was held off feed, whereas that from a pig fed 160 mcg. per pound of feed had activity when the pig was not taken off feed, and that from a pig fed 1280 mcg. per pound of feed had activity for either no time off feed or for only 24 hours off feed is interesting. It indicates that both the level of feeding and the time feed containing stilbestrol is removed before slaughter are involved in the estrogenic activity of liver tissue. These findings are not entirely surprising since Whiting et al. (84) found some activity in one out of two trials for the liver of lambs that had been implanted with stilbestrol at the start of the feeding period. Stob et al. (75) found activity in beef livers from steers implanted with stilbestrol, but none for the livers of similarly treated lambs. On the other hand, Burroughs et al. (24), and Preston et al. (68), found no activity in beef liver from steers that had been off feed as little as 24 hours.

Since the pigs in these investigations showed no

significant response in rate of gain or in feed efficiency we have no exact estimate of the correct level of stilbestrol for the pig, and of course make no recommendation for its use. However, the fact that the liver from pigs fed 10 mcg. of stilbestrol per pound of feed up to time of slaughter showed no estrogenic activity suggested that the stilbestrol was being inactivated as quickly as it reached the liver. Stilbestrol administered orally is known to enter the circulation via the portal system, Hanahan et al. (44) and Twombly and Schoenwaldt (82). The latter workers have also shown from work with injections of radioactive stilbestrol that the highest concentration of any organ occurs in the liver for the mouse, rabbit, and dog.

Unanswered, however, is the point of whether it is the low level from which the liver showed no estrogenic activity or the higher levels which did that corresponds to levels in other species which are stimulatory to rate of gain and feed efficiency.

The progesterone-estradiol implant may have possibilities with lambs, as ewe lambs so treated in Experiment 4 gained 15 percent more on 16 percent less feed, but the ratio of progesterone to estradiol is either incorrect or the does used was too large. This particular dose and ratio produced both growth and aging at the same time.

The growth was evident in greater gains but less finish, and the aging in the fact that 50 percent of the lambs yielded carcasses which were classed as yearlings because of the whiteness of the bones and complete ossification of the break joint. Then, too, the treatment stimulated sexual excitement and the mating reaction which Turner (80) suggests depends upon an optimal progesterone-estradiol ratio, at least in the guinea pig.

The work with the androgenic compounds, testosterone propionate and methyl testosterone, is difficult to explain in that a significant stimulation in rate of gain was produced in only one of four trials. In this, the implantation of testosterone propionate produced growth but lack of finish and enlargement of the thyroid, whereas the feeding of methyl testosterone produced more finish than the basal ration, no evidence of growth, and a reduction in the size of the thyroid - all differences being significant. Leonard (54) working with testosterone propionate in the castrate rat has shown that this androgenic compound increases the glycogen level of skeletal muscle and can exert this effect in the absence of the pituitary. Kochakain (53) has shown that the growth promoting and nitrogen retention action of the androgens is independent of the anterior pituitary. Both of the above suggest no

involvement of the thyroid, but do not rule out a change in basal metabolism. The fact that shorn lambs injected with testosterone propionate, in Experiment 4, outgained their wooled pen mates suggests a possible increase in metabolic rate and if this was true the shorn lambs may have spent less energy for the elimination of heat and more for gain, but this explanation would need be supported by increased feed intake and increased rate of gain by the shorn-implanted lambs as compared to the shorn lambs in the control group and such was not the case.

The feeding of methyl testosterone and stilbestrol, or the implanting of testosterone propionate in lambs being fed stilbestrol, produced only one observation of note. Either androgen prevented stilbestrol from exerting its usual effect in making the pelts difficult to remove. This same observation has been reported by O'Mary et al. (61). This is another bit of evidence suggesting that there may be an opportunity for various combinations of hormones if combinations can be worked out that will eliminate or hold in check the undesirable effects without eliminating the desired one. Progesterone injected simultaneously with estrin prevents the development of sexual skin in monkeys that estrin alone produces, Hisaw et al. (46) and preliminary work in this trial indicates that progesterone

may prevent the enlarged vulva from developing from the feeding of stilbestrol to the female pig, but numbers were too limited to make a valid estimate. Sleeth et al. (73) report that estradiol benzeate causes swelling of the vulva and mammary glands of pigs, but that the injecting of testosterone propionate simultaneously eliminated the effect. Testosterone propionate increased pelt weight in ewe lambs in Experiment 1 and Acker et al. (1) found that a progesterone-estradiol implant increased pelt weight in lambs. It is known that testosterone alone increases and that thyroxine alone decreases epidermal thickness in the castrate rat, Eartly et al. (35). Thus it would seem that the combinations that are possibilities are numerous and no doubt many will be tried until the mode of action of the synthetic sex hormones is better understood. Perhaps the work done thus far, with presently available compounds, is just a beginning, and new compounds, produced by organic synthesis, will replace many of those now in use in the feeding of livestock. Certainly compounds that could alter metabolism in livestock feeding other than either thyroid drugs or synthetic compounds with sex hormone action are not beyond the realm of possibility.

It is fully recognized that one is treading on dangerous ground when the known action of hormones in one species

is used as the basis of speculation on like action in another. The same is probably true for differences in age, sex, and perhaps breed within the same species. Environmental temperature, level of protein in the ration, vitamin and mineral supplementation, level of energy, and even length of day may be other factors involved in the response obtained. Season of the year may be more important than now thought in the response with lambs. However until we determine the mode of action of the various sex hormones in sheep and swine it seems that their effects in other species must be recognized. Perhaps when the mode of action of the sex hormones is ultimately defined it may be found that species usually thought of as being much alike in nutritional requirements are really very unlike in normal hormone balance and, hence, could not be expected to respond similarly to sex hormone stimulation.

SUMMARY

One hundred twenty pigs and 184 lambs were used in a study of five synthetic hormone compounds in the production of quality, wholesome pork and lamb, and in observing the effects of certain endocrine stimulants on glands and tissues of the body.

Levels of stilbestrol of 0, 5, 10, 20, 40, 80, 160, 320, 640, and 1280 mcg. per pound of ration were fed in two experiments to growing-finishing pigs from 33 to 200 pounds weight. Rate of gain, feed intake, and feed efficiency were not significantly affected by the feeding of stilbestrol, but levels of either 10, 80, or 160 mcg. per pound of feed increased rate of gain to a greater extent than the other levels fed. Live probe measurements, dressing percentage, and cooler shrink showed no significant differences due to treatment. Evidence of stimulation was shown in enlargement of the vulva and teats in gilts at levels of 160 mcg. or more of stilbestrol per pound of feed. These levels also increased the size of the rudimentary teats in barrows. Ovary size was depressed by stilbestrol in all but the 10 mcg. per pound level. Follicles were decreased in both size and number by stilbestrol. The diameter of the cervix and the weight of the reproductive tract were significantly

increased by treatment and the diameter of the cervix was correlated with level of feeding of stilbestrol.

Testosterone propionate, methyl testosterone, stilbestrol and a combination of progesterone and estradiol were either fed or implanted, singly and in various combinations to ewe lambs in four experiments. In the first trial, conducted in the December to March feeding period, an injection of 3.3 mg. of testosterone propionate per 100 pounds live weight per day increased rate of gain by 38 percent over control lambs. Methyl testosterone fed at levels of either 2.41 or 12.07 mg. per lamb per day increased gains by 16 percent. Feed efficiency favored the treatments with the lambs injected with testosterone propionate requiring 19 percent less feed per unit of gain. Carcass grades and dressing percentages were highest in the group fed the higher level of methyl testosterone and lowest in the group treated with testosterone propionate. Testosterone propionate significantly increased the size of the thyroid, whereas the higher level of methyl testosterone reduced thyroid size. In subsequent experiments these results could not be duplicated with ewe lambs.

In three trials conducted at slightly different seasons of the year and all using an 11.5 percent protein ration stilbestrol had either no effect or caused a significant

depression in feed intake and rate of gain. Levels of stilbestrol from 0.003 to 2.68 mg. per lamb per day were used.

A commercial implant containing progesterone and estradiol significantly increased rate of gain, reduced the feed required per unit of gain, and increased the daily consumption of corn, but not of total feed. This treatment lowered carcass grades significantly and 50 percent of the carcasses were classified as yearlings because of shape, the whiteness of the bones, and the ossification of the break joint. Excessive riding was evident in the group implanted with progesterone and estradiol by the fourth day after implantation and 20 percent of the lambs accepted a vasectomized ram at least once during the first half of the 71-day trial.

In Experiment 4, the progesterone-estradiol implant enlarged uterine size significantly and reduced the size and number of follicles, but had no effect on weight of the ovaries. Stilbestrol, on the other hand, increased uterine size, decreased ovary size, and was detrimental to follicle size and development. Testosterone propionate had no effect on uterine size, but increased both the weight of the ovaries and the number of follicles measuring 2 mm. or more in diameter. Shorn lambs implanted with 30 mg. of testosterone propionate gained 27 percent faster than their wooled pen mates, but failed to gain significantly more than either shorn or wooled control lambs.

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APPENDIX

Table 27. Analyses of variance of average daily gain and live probe - Swine Experiment 622

Source of variation	Degrees of freedom	Mean squares[a]			
		Average daily gain		Live probe	
		37.6 to 100 lb.	37.6 to 200 lb.	100 lb.	200 lb.
Replication	1	0.05590	0.20450	0.0020	0.0062
Treatment	9	0.01909	0.02084	0.0238	0.0358
Linear component	1	---	0.0034	0.0001	0.0497
Quadratic component	1	---	0.0403	0.0237	0.1751
Residual	7	---	0.02055	0.0272	0.0139
Replication X treatment	9	0.02750	0.00912	0.0089	0.0174
Sub-total	19				
Sex	1	0.02080	0.00960	0.0020	0.02021
Sex X treatment	9	0.02311	0.01304	0.0095	0.0325
Experimental error	10	0.02097	0.01702	0.0198	0.0512
Total	39				

[a]Mean squares not significant at P = .05 or less.

Table 28. Analyses of variance of feed required per 100 lb. gain and of cooler shrink and dressing percentage - Swine Experiment 622

Source of variation	Degrees of freedom	Mean squares[a]			
		Feed required per 100 lb. gain			
		37.6 to 100 lb.	37.6 to 200 lb.	Cooler shrink[b]	Dressing percent[b]
Replication	1	980.00	312.00	0.93	3.87
Treatments	9	158.00	257.00	0.33	3.36
Linear component	1	9.85	84.00	0.744	1.147
Quadratic component	1	227.36	94.56	0.985	2.930
Residual	7	1183.79	304.76	0.179	3.737
Replication X treatment	9	151.00	202.00	0.920	6.630
Total	19				

[a]Mean squares not significant at P = .05 or less.

[b]Comparison of carcass values for gilts only.

Table 29. Analyses of variance of other observable effects - Swine Experiment 622

Source of variation	Degrees of freedom	Mean squares			
		Diameter of cervix	Weight of reproductive tract	Weight of ovaries	Pelvic inlet
Replication	1	1.80	3,377.40	7.70	0.00
Treatments	9	49.08	3,536.34	4.29	0.396
Linear component 1		326.21[a]	10,927.67	32.07[a]	2.55[b]
Quadratic component 1		94.56	5,817.67	5.85	0.0668
Residual 7		3.04	2,154.60	0.11	0.1347
Replication X treatment	9	20.35	3,710.00	2.49	0.3270
Total	19				

[a]Mean squares significant at P = .01 or less.

[b]Mean squares significant at P = .05 or less.

Table 30. Average daily gain to 200 pounds - Swine Experiment 622[a]

Stilbestrol mcg./pound basal ration	Rep. 1			Rep. 2			Reps. 1 & 2	
	B[b]	G[b]	Average	B	G	Average	Total	Average
				(pounds)				
0	1.90	1.67	1.78	1.35	1.76	1.56	6.68	1.67
5	1.82	1.79[c]	1.80	1.69	1.68	1.68	6.98	1.74
10	1.92	1.70	1.81	1.84	1.52	1.68	6.98	1.74
20	1.88	1.69	1.78	1.63	1.49	1.56	6.69	1.67
40	1.64	1.61	1.62	1.62	1.62	1.62	6.49	1.62
80	1.66	1.73	1.70	1.72	1.58	1.65	6.69	1.67
160	1.88	1.84	1.86	1.81	1.89	1.85	7.42	1.86
320	1.77	1.86	1.82	1.72	1.54	1.63	6.89	1.72
640	1.92	1.89	1.90	1.65	1.70	1.68	7.16	1.79
1280	1.65	1.88	1.76	1.51	1.52	1.52	6.56	1.64
Average	1.80	1.76	1.78	1.65	1.63	1.64	6.85	1.71

[a]Each figure represents one pig.

[b]B = barrows; G = gilts.

[c]Estimated value.

Table 31. Feed per 100 pounds gain to 200 pounds - Swine Experiment 622

Stilbestrol mcg./pound basal ration	Replicate 1	Replicate 2	Total	Average
			(pounds)	
0	331	328	659	330
5	339	335	674	337
10	314	318	632	316
20	325	329	654	327
40	343	321	664	332
80	378	319	697	348
160	315	305	620	310
320	319	329	648	324
640	317	313	630	315
1280	325	330	655	328
Average	331	323	653	327

Table 32. Live probe measurements - Swine
Experiment 622

Stilbestrol mcg./pound basal ration	37.6 to 100 lb.[a]			37.6 to 200 lb.[b]		
	Barrows	Gilts	Average	Barrows	Gilts	Average
			(inches)			
0	0.7	0.6	0.7	1.3	1.4	1.3
5	0.8	0.7	0.8	1.5	1.5	1.5
10	0.7	0.7	0.7	1.6	1.5	1.6
20	0.8	0.8	0.8	1.5	1.4	1.5
40	0.8	0.8	0.8	1.4	1.6	1.6
80	0.6	0.7	0.7	1.7	1.5	1.6
160	0.8	0.7	0.7	1.7	1.3	1.5
320	0.7	0.7	0.7	1.4	1.4	1.4
640	0.6	0.6	0.6	1.4	1.5	1.5
1280	0.7	0.8	0.7	1.7	1.6	1.6
Average	0.7	0.7	0.7	1.5	1.5	1.5

[a]Four pigs per pen. Eight pigs per level.

[b]Two pigs per pen. Four pigs per level.

Table 33. Analyses of variance of average daily gain and live probe - Swine Experiment 637

Source of variation	Degrees of freedom	Mean squares[a]		
		Average daily gain		Live probe
		33 to 100 lb.	33 to 200 lb.	
Replication	2	0.1052	0.0574	0.0121
Treatments	9	0.0202	0.0137	0.0365
Replication X treatment	18	0.0209	0.0258	0.0229
Sub-total	29			
Sex	1	0.1581	0.1297	0.0482
Sex X treatment	9	0.3073	0.1143	0.0222
Remainder	18	0.2665	0.1463	0.0220
Total	57			

[a]Mean squares not significant at P = .05 or less.

Table 34. Analyses of variance of feed required per 100 pounds gain and of cooler shrink and dressing percentage - Swine Experiment 637

Source of variation	Degrees of freedom	Mean squares[a]			
		Feed required per 100 lb. gain			
		33 to 100 lb.	33 to 200 lb.	Cooler shrink[b]	Dressing percent[b]
Replication	2	430.00	2241.00	0.085	2.37
Treatments	9	482.67	730.89	0.153	1.789
Linear component	1	---	---	0.0037	4.110
Quadratic component	1	---	---	0.0367	1.765
Residual	7	---	---	0.1913	1.460
Replication X treatment	16	235.56	464.56	0.0920	3.990
Total	27				

[a]Mean squares not significant at P = .05 or less.

[b]Comparison of carcasses for gilts only.

Table 35. Analyses of variance of other observable effects - Swine Experiment 637

Source of variation	Degrees of freedom	Mean squares			
		Diameter of cervix	Weight of reproductive tract	Weight of ovaries	Pelvic inlet
Replication	2	19.44	16,225.95[a]	2.09	0.10
Treatments	9	43.71	6,886.53	9.16[a]	0.222
Linear component	1	350.43	18,414.65[a]	64.08[a]	0.031
Quadratic component	1	17.33[a]	9,469.13	11.05[b]	0.384
Residual	7	3.66	4,870.72	1.05	0.226
Replication X treatment	17	21.45	3,839.23	1.77	0.1159
Total	28				

[a]Mean squares significant at P = .01 or less.

[b]Mean squares significant at P = .05 or less.

Table 36. Average daily gain to 200 pounds - Swine Experiment 637[a]

Stilbestrol mcg./pound basal ration	Replicate 1		2		3		Total	Average
	B[b]	G[b]	B	G	B	G		
	(pounds)							
0	1.62	1.66	1.68	1.56	1.67	1.60	9.79	1.63
5	1.69	1.55	1.79	1.59	1.71	1.56[c]	9.89	1.65
10	1.97	1.78	1.54	1.47	1.58	1.41	9.75	1.62
20	1.58	1.59	1.74	1.59	1.59	1.40	9.49	1.58
40	1.70[c]	1.73	1.45	1.44	1.67	1.68	9.67	1.61
80	1.86	1.50	1.96	1.64	1.79	1.67	10.42	1.74
160	1.76	1.98	1.47	1.41	1.90	1.57	10.09	1.68
320	1.63	1.65	1.54	1.71	1.56	1.49	9.58	1.60
640	1.73	1.67	1.49	1.51	1.58	1.51	9.49	1.58
1280	1.72	1.51	1.64	1.62	1.79	1.56	9.84	1.64
Average	1.73	1.66	1.63	1.55	1.68	1.54	9.80	1.63

[a]Each figure represents one pig.

[b]B = barrows; G = gilts.

[c]Estimated values.

Table 37. Feed per 100 pounds gain to 200 pounds - Swine Experiment 637[a]

Stilbestrol mcg./pound basal ration	Replicate 1	2	3	Total	Average
			(pounds)		
0	388	377	344	1109	370
5	386	372	353[b]	1111	370
10	357	377	387	1121	374
20	387	353	376	1116	372
40	385[b]	408	341	1134	378
80	369	356	341	1066	355
160	350	385	338	1073	358
320	344	400	340	1084	361
640	447	410	376	1233	411
1280	382	392	359	1133	378
Average	379.5	383.0	355.5	1118.0	372.7

[a]Each figure represents an average of two pigs.

[b]Estimated values.

Table 38. Live probe at 200 pounds - Swine Experiment 637

Stilbestrol mcg./pound basal ration	Replication 1		2		3		Total	Average
	B[a]	G[a]	B	G	B	G		
	(inches)							
0	1.5	1.6	1.6	1.5	1.6	1.6	9.4	1.6
5	1.6	1.4	1.6	1.6	1.8	1.6	9.6	1.6
10	1.6	1.6	1.1	1.4	1.6	1.4	8.7	1.4
20	1.5	1.2	1.9	1.4	1.6	1.4	9.0	1.5
40	1.6	1.5	1.6	1.4	1.6	1.8	9.5	1.6
80	1.8	1.5	1.6	1.8	1.7	1.6	10.0	1.7
160	1.6	1.6	1.5	1.4	1.7	1.4	9.2	1.5
320	1.3	1.4	1.6	1.6	1.4	1.4	8.7	1.4
640	1.6	1.2	1.3	1.6	1.4	1.4	8.5	1.4
1280	1.6	1.6	1.3	1.6	1.6	1.6	9.3	1.6
Average	1.6	1.5	1.5	1.5	1.6	1.5	9.2	1.5

[a]B = barrows; G = gilts.

Table 39. Feed consumed per day - Swine Experiment 637[a]

Stilbestrol mcg./pound basal ration	Replicate 1	2	3	Total	Average
			(pounds)		
0	6.37	6.10	5.62	18.09	6.03
5	6.26	6.27	5.77[b]	18.30	6.10
10	6.68	5.69	5.77	18.14	6.05
20	6.13	5.88	5.61	17.62	5.87
40	6.10[b]	5.54	5.70	17.34	5.78
80	6.16	6.35	5.89	18.40	6.13
160	6.51	5.55	5.81	17.87	5.96
320	5.65	6.50	5.19	17.34	5.78
640	7.62	6.13	5.80	19.55	6.52
1280	6.18	6.38	5.98	18.54	6.18
Average	6.37	6.04	5.71	18.19	6.06

[a]Each figure represents an average of two pigs.

[b]Includes one estimated value.

Table 40. Analyses of variance of uterine weights in pork liver and fat assay - Swine Experiment 637 (standard compared to unknown from pigs fed stilbestrol)

Source of variation	Degrees of freedom	Mean squares: Liver 160 mcg./lb. level not off feed	Liver 160 mcg./lb. level off feed 24 hrs.	Fat 1280 mcg./lb. level not off feed	Fat 160 mcg./lb. level not off feed
Replicates	1	21.06	0.63	80.65	1.08
Standard vs. unknown	1	276.67[a]	10.25	36.09	22.96
Linear regression	1	420.17[a]	958.10[a]	2676.73[a]	1838.21[a]
Parallelism	1	7.00	0.139	135.34	7.67
Quadratic regression	1	0.0005	25.92	0.867	72.23
Difference of quadratic	1	12.28	4.45	22.09	22.00
Error	5	7.63	18.07	42.10	81.76
Total	11				

[a]Mean squares significant at P = .01 or less.

Table 41. Analyses of variance of uterine weights in pork liver assay - Swine Experiment 637 (standard compared to unknown from pigs fed 1280 mcg. of stilbestrol per pound of ration and off feed 0, 24, and 48 hours before slaughter)

Source of variation	Degrees of freedom	Hours off feed containing stilbestrol		
		0	24	48
		Mean squares	Mean squares	Mean squares
Replicates	1	2.17	12.41	21.34
Standard vs. unknown	1	5372.10[a]	3411.79[a]	7.36
Linear regression	1	224.25[a]	424.37[a]	481.11[a]
Parallelism	1	8.25	99.62	2.45
Quadratic regression	1	39.61	22.77	7.07
Difference of quadratic	1	23.53	3.14	0.00875
Error	5	12.90	7.19	16.28
Total	11			

[a]Mean squares significant at P = .01 or less.

Table 42. Analyses of variance of weight gains, feed data, and carcass characteristics - Lamb Experiment 1

Source of variation	Degrees of freedom	Av. daily gain	Av. feed per day	Feed per 100 lb. gain	Cooler shrink	Dressing percent	Carcass grade	Percent fat in 3 rib cut	Pelt weight
Replication	3	216.35	0.15	1.587	0.937	1.23	0.396	7.73	1.01
Treatment	3	82.59[a]	0.156	0.99	2.800	5.84	3.560[a]	29.61	3.90
Replication X treatment	9	6.89	0.134	1.31	1.160	2.32	0.396	31.81	1.46
Total	15								

[a]Mean square significant at P = .01.

Table 43. Analyses of variance of weight of various organs of the body - Lamb Experiment 1

Source of variation	Degrees of freedom	Mean squares				
		Liver	Thyroid	Adrenals	Uterus	Ovaries
Replication	3	10,123.42	0.66	0.173	0.025	0.0265
Treatment	3	20,010.75	3.94[a]	0.430	0.130	0.130
Replication X treatment	9	8,332.36	0.86	0.430	0.062	0.061
Total	15					

[a]Mean square significant at P = .05.

Table 44. Analyses of variance of weight gain and feed data, carcass grade, and of weights of uteri and ovaries - Lamb Experiment 2

Source of variation	Degrees of freedom	Mean squares[a] Average daily gain	Feed required per 100 lb. gain	Carcass grade	Weight of uteri	Weight of ovaries
Replication	3	35.01	0.457	4.25	19.39	0.029
Treatment	7	22.62	2.31	2.07	64.53	0.661
Replication X treatment	21	39.20	4.45	2.11	26.37	0.665
Total	31					

[a]Mean squares not significant at P = .05 or less.

Table 45. Analyses of variance of average daily gain, cooler shrink, uterine weights, ovary weights, and number of follicles - Lamb Experiment 3

Source of variation	Degrees of freedom	Mean squares: Average daily gain	Cooler shrink	Weight of uteri	Weight of ovaries	Number of follicles
Replication	3	0.0025	0.336	40.53	0.084	0.644
Treatment	7	0.0106	0.674	135.11[a]	0.216	1.04[a]
Replication X treatment	21	0.0043	0.747	10.19	0.090	0.218
Total	31					

[a]Mean squares significant at P = .01.

Table 46. Analyses of variance of average daily gains, cooler shrink, carcass grades, weight of uteri, and weight of ovaries - Lamb Experiment 4

Source of variation	Degrees of freedom	Mean squares: Average daily gain	Cooler shrink	Carcass grade	Weight of uteri	Weight of ovaries
Replication within wool groups	18	0.55	0.028	0.83	1.93	0.029
Wool	1	0.36	0.770	0.16	160.98	0.360
Rations	4	4.15[a]	1.220	30.53[a]	1010.17[a]	1.0375[a]
Rations X wool	4	0.27	0.200	0.405	21.66	0.0675
Replication X treatment	68	13.51	0.503	0.0306	61.45	0.196
Total	95					

[a]Mean squares significant at P = .05 or less.

Table 47. Average daily gains and feed consumption for individual lambs - Lamb Experiment 1

Group	Average daily gain				Average daily feed intake			
	(pounds)							
1	0.40	0.45	0.37	0.31	3.38	3.48	2.94	2.96
2	0.46	0.31	0.51	0.49	3.71	3.27	3.67	3.48
3	0.36	0.40	0.45	0.49	3.36	3.19	3.82	4.00
4	0.49	0.31	0.64	0.59	3.68	2.86	4.15	3.77

Table 48. Average daily gains and feed consumption for individual lambs - Lamb Experiment 2

Group	Average daily gain				Average daily feed intake			
	(pounds)							
1	0.39	0.37	0.16	0.35	3.48	3.63	2.82	3.52
2	0.37	0.28	0.47	0.41	3.39	3.32	3.55	3.95
3	0.45	0.36	0.28	0.37	4.22	3.30	2.70	3.70
4	0.24	0.43	0.41	0.41	3.12	3.71	3.54	3.52
5	0.45	0.44	0.31	0.48	3.67	3.65	3.13	4.16
6	0.43	0.26	0.35	0.40	4.04	3.26	3.48	3.79
7	0.27	0.40	0.36	0.43	3.24	3.55	3.30	3.87
8	0.37	0.37	0.38	0.41	3.31	3.63	3.61	3.96

Table 49. Average daily gains and feed consumption for individual lambs - Lamb Experiment 3

Group	Average daily gain						Average daily feed intake					
	(pounds)											
1	0.38	0.50	0.51	0.42	0.35	0.53	3.42	3.69	4.01	4.29	3.13	3.69
2	0.29	0.40	0.38	0.25	0.40	0.33	2.93	3.78	3.28	2.92	3.74	3.24
3	0.36	0.32	0.45	0.38	0.45	0.29	3.33	2.94	3.55	3.37	3.79	2.64
4	0.41	0.33	0.45	0.40	0.42	0.38	2.95	3.10	4.52	3.82	3.52	3.37
5	0.47	0.36	0.41	0.41	0.36	0.38	3.20	3.45	3.66	3.27	3.13	3.32
6	0.41	0.58	0.41	0.45	0.45	0.40	3.51	3.96	3.44	3.59	3.61	3.55

Table 50. Average daily feed per lamb and feed required per 100 pounds gain - Lamb Experiment 4

Group	Treatment	Average feed per day				Feed required per 100 lbs. gain			
		Cracked corn	Soybean oil meal	Alfalfa hay	Total	Cracked corn	Soybean oil meal	Alfalfa hay	Total
		(pounds)							
1	Control	1.51	0.25	1.49	3.25	311.17	51.51	306.43	699.11
2	1.4 mg. stilbestrol per lamb per day	1.41	0.25	1.44	3.10	334.17	59.17	341.10	734.44
3	2.67 mg. stilbestrol per lamb per day	1.36	0.25	1.39	3.00	322.52	59.33	330.44	712.29
4	Progesterone-estradiol implant	1.56	0.25	1.44	3.25	282.06	45.33	261.43	588.82
5	30 mg. testosterone propionate implant	1.48	0.25	1.45	3.18	324.23	54.62	316.54	695.39

Table 51. Summary of daily gains per lamb by treatments and within treatments by wool groups - Lamb Experiment 4

Group 1 Basal		Group 2 Stilbestrol 1.4 mg./day		Group 3 Stilbestrol 2.67 mg./day		Group 4 Progesterone-estradiol		Group 5 Testosterone propionate	
W	S	W	S	W	S	W	S	W	S
				(pound)					
.54	.54	.37	.55	.49	.25	.63	.70	.41	.62
.46	.46	.21	.32	.35	.38	.68	.54	.45	.54
.34	.54	.55	.61	.44	.56	.70	.59	.32	.52
.41	.37	.30	.49	.45	.55	.45	.58	.54	.66
.48	.62	.58	.44	.37	.28	.41	.44	.38	.70
.55	.52	.39	.42	.44	.44	.54	.59	.30	.28
.45	.62	.48	.35	.48	.34	.42	.49	.41	.38
.54	.41	.58	.42	.32	.51	.49	.58	.32	.52
.38	.51	.42	.28	.45	.42	.58	.66	.42	.45
.51	.46	.32	.37	.39	.42	.55	.41	.45	.48
				Average					
.47	.50	.42	.42	.42	.41	.55	.56	.40	.52